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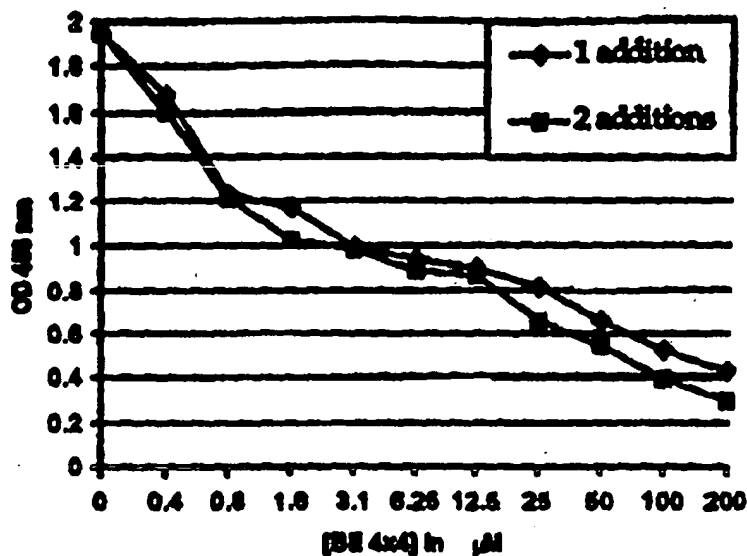
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(54) Title: DELIVERY OF THERAPEUTIC AGENTS TO THE PROSTATE

(57) Abstract

Novel methods for delivery of pro-apoptotic and/or anti-proliferative agents to the prostate gland for treatment of prostate disorders are disclosed. Kits for practicing the methods of the invention are also provided. The methods and kits of the invention can employ a catheter-based delivery system that uses phoresis, pressure, or both, to assist in the delivery of the pro-apoptotic and/or anti-proliferative agents to the prostate gland and/or surrounding regions.



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DELIVERY OF THERAPEUTIC AGENTS TO THE PROSTATE

10

Technical Field

The invention relates to materials, methods and kits for treatment of disorders of the prostate, including benign prostatic hyperplasia (BPH). More specifically, the invention relates to methods and kits for treating disorders of the prostate by delivery of pro-apoptotic and/or anti-proliferative agents to the prostate and/or periprostatic space.

20 Background

Disorders of the prostate gland such as benign prostatic hyperplasia (BPH) or localized prostate cancer have been treated by a variety of methods with limited success, including treatment by oral administration of various drugs, surgical procedures such as transurethral resection or open prostatectomy, and radiation targeted to the prostate region. Oral medications for the treatment of these disorders have been suboptimal with respect to both efficacy and toxicity. Surgical procedures and radiation therapy involving the prostate gland, while occasionally effective, are much more inconvenient than medical therapy and are often associated with even more morbid side effects than are medications.

Although many prostatic disorders exist, two of the most prevalent and medically significant are BPH and localized prostate cancer. BPH is a common

prostatic disorder in men which becomes clinically manifest usually after age fifty. In BPH, hyperplastic growth of prostatic cells in the periurethral glandular tissue in the central zone of the prostate gland causes an enlarged prostate which can compress or elongate the urethra and produce symptoms of urethral obstruction that may progress to urinary retention or to a constellation of symptoms known as prostatism. These conditions include reduced urinary flow, hesitancy in initiating voiding, postvoid dribbling, a sensation of incomplete bladder emptying, and in extreme cases, the development of bladder or higher urinary tract infections.

BPH has been treated medically or surgically with some success using techniques such as partial prostatectomy, transurethral resection of the prostate (TURP), transurethral incision of the bladder neck, balloon dilatation of the prostate, urinary diversion by catheter, laser therapy, laser prostatectomies, hyperthermia, ultrasonic and radiofrequency ablation, transurethral microwave thermal therapy using a catheter (U.S. Patent No. 5,464,437 to Reid et al.), and treatment with hydrolytic enzyme compositions (U.S. Patent No. 5,116,615 to Gokcen & Guy). See, e.g., *Cecil Textbook of Medicine*, 19th ed., 1353 (Wynngaarden et al., eds., W.B. Saunders 1992). Such medical or surgical therapies almost always result in some degree of attendant tissue trauma, and have been associated with a wide range of complications such as bleeding, impotence, bladder neck contracture, incontinence, urethral stricture, metal stenosis, fistula formation, bowel dysfunction, and prostatitis.

Pharmacological treatments of BPH have included systemic administration of finasteride (Proscar) and terazosin (Hytrin). Finasteride reduces the levels of dihydrotestosterone, the active metabolite in the prostate, causing shrinkage of the

prostate and relief of symptoms. Terazosin is an α -1-selective blocker that relaxes smooth muscle in the prostate and bladder. Antiandrogen therapy has also been used to atrophy the prostatic epithelium. These
5 medical therapies do not cure BPH, are not always effective, have some toxic side effects, can be slow to act, and often need to be chronically administered for maximum efficacy.

Accordingly, there remains a need to provide
10 an effective and practical method for treating prostatic disorders. It would also be advantageous to develop a system of administration of pharmaceutical agents directly to the prostate, in order to avoid the complications discussed above from medical, surgical
15 or other methods of treating prostatic disorders.

Summary of the Invention

In one embodiment, a method for treating a prostatic disorder in a mammalian subject is provided.
20 The method entails delivery of a pharmaceutical composition containing a therapeutically effective amount of a pro-apoptotic agent to the prostate of the subject.

In related aspects of the method, the pro-apoptotic agent is selected from the group consisting
25 of phenyl buterate, phenyl acetate, 1-phenyl-2-decanoyl amino-3-morpholino-1-propanol (PDMP), thapsigargin, 4-substituted-1,2-naphthoquinones, and pharmaceutically acceptable salts thereof. In other
30 related aspects of the method, the pro-apoptotic agent is a polypeptide selected from the group consisting of fas, fas ligand, fadd, fap-1, tradd, faf, rip, reaper, apoptin, interleukin-2 converting enzyme (ICE), FADD-homologous ICE-CED3-like protease (FLICE), bax, P53,
35 TNF receptor type-I, TNF-alpha, and cytochrome-c. Alleles, variants and functionally active fragments of

the above polypeptide pro-apoptotic agents may also be used in the present methods.

In another embodiment, a method for treating a prostatic disorder in a mammalian subject is provided. The method entails delivery of a pharmaceutical composition containing a therapeutically effective amount of an anti-proliferative agent to the prostate of the subject. In a related aspect of the invention, the anti-proliferative agent is a polyamine analog.

In yet another embodiment of the invention, a method for treating a prostatic disorder in a mammalian subject is provided, wherein the method entails delivering to the prostate of the subject a therapeutically effective amount of a polynucleotide molecule comprising a coding sequence for a pro-apoptotic agent operably linked to control elements, whereby said coding sequence can be transcribed and translated in the subject *in vivo*.

In related aspects of the method, the coding sequence encodes a pro-apoptotic agent selected from the group consisting of fas, fas ligand, fadd, fap-1, tradd, faf, rip, reaper, apoptin, interleukin-2 converting enzyme (ICE), FADD-homologous ICE-CED3-like protease (FLICE), bax, P53, TNF receptor type-I, TNF-alpha, and cytochrome-c.

In a still further embodiment of the invention, a kit is provided for use in treating a prostatic disorder in a mammalian subject. The kit contains a pharmaceutical composition containing a therapeutically effective amount of a pro-apoptotic and/or an anti-proliferative agent and instructions for treating the subject using the pharmaceutical composition.

Brief Description of the Figures

Figure 1 depicts the results of the *in vitro* cytotoxicity assay described in Example 3, wherein a polyamine analog pro-apoptotic agent was administered to the human prostate cancer cell line DU-145.

Detailed Description of the Invention

The practice of the present invention may employ conventional methods of immunology, microbiology, molecular biology and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, et al. *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *DNA Cloning: A Practical Approach*, vol. I & II (D. Glover, ed.); *Oligonucleotide Synthesis* (N. Gait, ed., 1984); *Nucleic Acid Hybridization* (B. Hames & S. Higgins, eds., 1985); *Transcription and Translation* (B. Hames & S. Higgins, eds., 1984); *Animal Cell Culture* (R. Freshney, ed., 1986); Perbal, *A Practical Guide to Molecular Cloning* (1984); and *Handbook of Experimental Immunology*, Vols. I-IV (D.M. Weir and C.C. Blackwell eds., 1986, Blackwell Scientific Publications).

As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise.

I. Definitions

As used herein, the term "prostatic disorder" refers to any disorder related to the prostate, excluding any cancerous condition of the prostate. Benign prostatic hyperplasia (BPH) is one particular prostatic disorder that can be treated with the methods of the invention. Therefore, the disorder may be known or unknown, have a biological and/or medical basis, and be characterized by mild or severe

symptoms. Diagnosis of a particular prostatic disorder can be based on vague or specific symptoms that are either local or systemic. The prostatic disorder being treated in the practice of the invention may be part of a larger, more generalized condition or disorder occurring in the subject. Prostatic disorders may be assessed, monitored and/or detected by physical exam and other non-invasive diagnostic procedures including radionuclide imaging, positron emission tomography, magnetic resonance imaging, and the like.

The terms "pharmaceutical agent," and "pharmaceutically active agent," as used herein, encompass any compound or composition of matter which, when administered to a vertebrate subject, induces a desired pharmacologic and/or physiologic effect by local and/or systemic action. The term therefore encompasses those compounds or chemicals traditionally regarded as drugs, vaccines and biopharmaceuticals, including, without limitation: proteins, peptides and fragments thereof (whether naturally occurring, chemically synthesized or recombinantly produced); peptoids; radionuclides; small molecules; nucleic acid molecules (polymeric forms of two or more nucleotides, either ribonucleotides (RNA) or deoxyribonucleotides (DNA) including both double- and single-stranded molecules, gene constructs, and expression vectors); carbohydrates; polysaccharides; and the like. In the practice of the invention, the pharmaceutical agent will be selected using such parameters as the nature of the prostatic disorder being prevented, ameliorated or treated, a general and specific diagnosis of the subject being treated, and the particular goals of the therapy.

"Pharmaceutically acceptable salts" refer to those salts which retain the biological effectiveness and properties of the base compounds and which are not

biologically or otherwise undesirable.

Pharmaceutically acceptable salts of a pharmaceutical agent existing in acid form may be prepared using conventional methods by treating the free acid with at least one molar equivalent of a pharmaceutically acceptable base, e.g., sodium hydroxide, potassium hydroxide, ammonium hydroxide, calcium hydroxide, trimethylamine, and the like. Pharmaceutically acceptable salts of a pharmaceutical agent existing in base form may be prepared using conventional methods by treating the free base with at least one molar equivalent of a pharmaceutically acceptable organic or inorganic acid, e.g., phosphoric acid, hydrochloric acid, sulfuric acid, hydrobromic acid, acetic acid, propionic acid, glycolic acid, oxalic acid, malonic acid, succinic acid, tartaric acid, cinnamic acid, methane sulfonic acid, p-toluene sulfonic acid, salicylic acid, and the like.

The term "pro-apoptotic agent" encompasses any pharmaceutical agent that can induce apoptosis or programmed cell death in a cell. Pro-apoptotic agents for treatment of prostatic disorders are known, and include those particular pharmaceutical agents described herein.

The term "anti-proliferative agent" encompasses pharmaceutical agent that can arrest cell proliferation and/or cell growth in a cell or population of cells. The arrest of cell proliferation can be permanent or temporary, and may or may not kill the affected cells. The term includes agents commonly referred to as chemotherapeutic agents.

By "therapeutically effective" amount is meant a nontoxic but sufficient amount of a pharmaceutically active agent to provide a desired preventive, ameliorative, and/or curative therapeutic effect. A precise therapeutically effective amount for a subject will depend upon the subject's size and

health, the nature and extent of the prostatic condition, and the particular agent selected for administration. The effective amount for a given situation can be determined by routine experimentation
5 by one of skill in the art.

The terms "carriers" or "vehicles" are used herein to refer to materials suitable for administration of a pharmaceutical agent, and include any such materials known in the art, e.g., any liquid,
10 gel, solvent, liquid diluent, solubilizer, or the like, which is nontoxic and which does not interact with other components of the composition in a deleterious manner. A "pharmaceutically acceptable carrier" is any pharmaceutical carrier that does not
15 itself cause undue adverse reactions.

Examples of suitable carriers for use herein include water, silicone, liquid sugars, waxes, petroleum jelly, glycerol, ethanol, and a variety of other materials. Auxiliary substances, such as
20 wetting or emulsifying agents, pH buffering substances, and the like, may also be present. Liposomes are included within the definition of a pharmaceutically acceptable carrier. Suitable liposome compositions are described in U.S. Patent No.
25 5,422,120, International Publication Nos. WO 95/13796, WO 94/23697, and WO 91/14445, and in EP 524,968 B1.

Particular carriers may be more appropriate for various modes of administrations. For example, ion-free carriers are more efficient for iontophoretic
30 administration of pharmaceutical agents. In situations where administration is conducted without the use of iontophoresis, carriers including mineral acids, salts (e.g., hydrochlorides, hydrobromides, phosphates, sulfates), and salts of organic acids
35 (e.g., acetates, propionates, malonates, and benzoates and the like) can be used. A thorough discussion of pharmaceutically acceptable excipients is available in

Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

The terms "transurethral," "intraurethral," or "urethral," are used herein interchangeably to refer to delivery of a pharmaceutical agent into the urethra such that drug contacts and passes through the wall of the urethra.

When pharmaceutical compositions are administered transurethrally, the compositions may contain one or more permeation enhancers, i.e., compounds which act to increase the rate at which the selected agent permeates through the urethral membrane. Examples of suitable permeation enhancers include dimethylsulfoxide (DMSO), dimethylformamide (DMF), propylene glycol, lecithin, alcohols, or the like. The pharmaceutical compositions may additionally include one or more enzyme inhibitors designed to inhibit any degrading enzymes which may be present in the urethra. Such enzyme inhibiting compounds may be determined by those skilled in the art by reference to the pertinent literature and/or by using routine experimental methods.

"Phoresis" refers to a class of techniques that employs the principles of charge separation of differentially charged molecules in an electric field. Phoresis can be used with transurethral delivery techniques and other delivery methods, and includes techniques such as iontophoresis, electrophoresis, phonophoresis, and sonophoresis.

"Iontophoresis," as used herein, refers to use of an electrical current to administer electrically charged and uncharged pharmaceutical agents. Examples of iontophoresis methodology are described in U.S. Pat. No. 4,411,648 and U.S. Pat. No. 5,222,936, U.S. Pat. No. 5,286,254; and WO 94/05369.

The term "electrophoresis" as used herein refers to a technique for separating charged molecules

in buffer solution based on their different mobilities in an electric field generated by direct current through the buffer.

"Phonophoresis" or "sonophoresis" refers to separation of molecules or particles based on the principles of ultrasound, using ultrasonic electrical transduction to create an ultrasonic field that promotes delivery of pharmaceuticals to tissues in the body where a phoretic catheter is positioned. The functioning of a phonophoretic catheter is described in U.S. Patent No. 5,286,254.

The term "catheter" generally refers to a tubular, flexible, surgical instrument that can be inserted into a cavity of the body for drainage of body fluids, or administration of pharmaceutical agents. Common catheter placement locations include veins, arteries and the bladder, especially to promote urine drainage. Increased efficiency of delivery of pharmaceutical agents by catheter can be achieved by the use of pressure. Pressure can be used alone, or in combination with phoresis, e.g., in combination with iontophoresis, in order to effect delivery of a pharmaceutical agent to the prostate, periprostate tissue, or surrounding tissue. Heat may also be used in conjunction with a catheter-based delivery protocol.

The term "chasing agent" refers to any non-toxic agent that can be used in pressure-assisted catheter delivery of a pharmaceutical agent for pushing the agent through the urethra, or other tissue near the prostate, to the prostate. Suitable non-toxic chasing agents include, for example, saline, polyethylene glycol (PEG), or the like.

By "mammalian subject" is meant any member of the class *Mammalia*, including, without limitation, humans and other primates, including such non-human primates as chimpanzees and other apes and monkey

species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; and laboratory animals including rodents such as mice, rats and guinea pigs. The term does not denote a particular age or sex. Thus, both adult and newborn individuals, as well as fetuses, either male or female, are intended to be covered.

As used herein, the term "polypeptide" refers to a polymer of amino acids and does not refer to a specific length of the product; thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide. This term also does not refer to, or exclude, post-translational modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

By "purified" and "isolated" is meant, when referring to a polypeptide or nucleotide sequence, that the indicated molecule is present in the substantial absence of other biological macromolecules of the same type. The term "purified" as used herein preferably means at least 75% by weight, more preferably at least 85% by weight, more preferably still at least 95% by weight, and most preferably at least 98% by weight, of biological macromolecules of the same type are present. An "isolated nucleic acid molecule which encodes a particular polypeptide" refers to a nucleic acid molecule which is substantially free of other nucleic acid molecules that do not encode the subject polypeptide; however, the molecule may include additional bases or moieties which do not deleteriously affect the basic

characteristics of the composition. Thus, for example, an isolated nucleic acid molecule which encodes a particular pro-apoptotic agent consists essentially of the nucleotide coding sequence for the subject agent.

"Homology" refers to the percent of identity between two polynucleotide or polypeptide moieties. The correspondence between the sequence from one form to another can be determined by techniques known in the art. For example, homology can be determined by a direct comparison of the sequence information between two polypeptide molecules. Alternatively, homology can be determined by hybridization of polynucleotides under conditions which provide for the formation of stable duplexes between homologous regions (for example, those which would be used prior to S_1 digestion), followed by digestion with single-stranded specific nuclease(s), followed by size determination of the digested fragments. Two DNA or polypeptide sequences are "substantially homologous" when at least about 80% (preferably at least about 90%, and most preferably at least about 95%) of the nucleotides or amino acids match over a defined length of the molecule.

A "biologically active fragment" refers to protein or polypeptide fragments that retain one or more of the activities of the full-length protein. With reference to a protein or polypeptide pharmaceutical in the context of the invention, and gene products produced by gene therapy, it is contemplated that such protein or polypeptides include biologically active fragments, truncations, variants, alleles, analogs and derivatives thereof. Unless specifically mentioned otherwise, such truncations, variants, alleles, analogs, and derivatives possess one or more of the bioactivities of the native mature protein. This term is not limited to a specific

length of the product of the gene. Thus, polypeptides that are identical or contain at least 60%, preferably 70%, more preferably 80%, and most preferably 90% sequence identity to the native protein fragment or
5 the native mature protein, wherever derived, from human or nonhuman sources are included within the term polypeptide.

The terms "alleles" and "variants" refer to polypeptides that differ from a native specified
10 protein by virtue of one or more amino acid substitutions, deletions, or insertions. The amino acid substitutions can be conservative amino acid substitutions or substitutions to eliminate non-essential amino acid residues, such as to alter a
15 glycosylation site, a phosphorylation site, an acetylation site, or to alter the folding pattern by altering the position of the cysteine residue that is not necessary for function, etc. Conservative amino acid substitutions are those that preserve the general
20 charge, hydrophobicity/hydrophilicity and/or steric bulk of the amino acid substituted, for example, substitutions between the members of the following groups are conservative substitutions: Gly/Ala, Val/Ile/Leu, Asp/Glu, Lys/Arg, Asn/Gln, Ser/Cys/Thr,
25 and Phe/Trp/Tyr.

The terms "nucleic acid molecule," and "polynucleotide molecule," refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. These terms refer only to
30 the primary structure of the molecule and thus include double- and single-stranded DNA and RNA. The terms also encompass known types of modifications, for example, labels which are known in the art, methylation, "caps", substitution of one or more of
35 the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl

phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example

5 proteins (including for e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.),

10 those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide.

A "coding sequence" is a nucleic acid molecule which is translated into a polypeptide,

15 usually via mRNA, when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence may be determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can

20 include, but is not limited to, cDNA, and recombinant nucleotide sequences.

"Control sequence" refers to nucleic acid sequences which are necessary to effect the expression of coding sequences to which they are ligated. The

25 nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences

30 include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is necessary for expression of a coding sequence, and may also include additional components whose presence

35 is advantageous, for example, leader sequences and fusion partner sequences.

"Operably linked" refers to a juxtaposition of elements wherein the components so described are in a relationship permitting them to function in their intended manner. For example, a control sequence

5 "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

By "vector" is meant any genetic element,

10 such as a plasmid, phage, transposon, cosmid, chromosome, virus etc., which is capable of replication when associated with the proper control elements and which can transfer gene sequences between cells. Thus, the term includes cloning and expression

15 vehicles, as well as viral vectors.

"Transfection" refers to the uptake of foreign DNA by a cell, and a cell has been "transfected" when exogenous DNA has been introduced inside the cell membrane. In this manner, the

20 exogenous DNA may or may not be integrated (covalently linked) to chromosomal DNA making up the genome of the cell. In procaryotes and yeasts, for example, the exogenous DNA can be maintained on an episomal element, such as a plasmid. With respect to the

25 invention, a eucaryotic cell is "stably transfected" when exogenous DNA has become integrated into the cellular genome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eucaryotic cell

30 to establish cell lines or clones comprised of a population of daughter cells containing the exogenous DNA. "Transient transfection" refers to cases where exogenous DNA does not remain in the cells for an extended period of time, e.g., where plasmid DNA is

35 transcribed into mRNA and translated into protein without integration into the host cell genome.

"Gene transfer" or "gene delivery" refers to methods or systems for reliably inserting foreign DNA into host cells such that the transferred genetic material is stable with respect to the loci of
5 insertion and is also susceptible to expression by the host cells. Such methods provide a unique approach for the treatment of acquired and inherited diseases. A number of systems have been developed for gene transfer into mammalian cells. See, e.g., U.S. Patent
10 No. 5,399,346.

II. General Methods

The invention relates to methods for delivering pro-apoptotic and/or anti-proliferative
15 agents to the prostate to treat prostatic disorders. The methods entail delivery of a variety of agents by conventional modes of administration or by gene therapy. Preferred modes of administration include catheter-based delivery to the urethra, with or
20 without the assistance of pressure or phoretic methods.

In the practice of the invention, pharmaceutical agents can be administered alone, or in combination with other agents to bring about a desired
25 therapeutic effect for the prevention, amelioration or treatment of a prostatic condition. Combined agents may be administered in the same, or in separate, pharmaceutical compositions.

Combination therapies are useful in the treatment of prostatic conditions that respond more
30 favorably to two or more agents when administered together. Administration of a combination of agents may be simultaneous or consecutive, and successive administrations may follow at any appropriate time
35 interval.

The pro-apoptotic and anti-proliferative agents are prepared and delivered to a subject as described herein below.

5 (A). Preparation of Pro-Apoptotic Compounds and
 Compositions

 A number of suitable pro-apoptotic compounds and compositions can be readily prepared for use with the present invention. For example, phenyl buterate,
10 phenyl acetate, 1-phenyl-2-decanoyl amino-3-morpholino-1-propanol (PDMP) and other inhibitors of glucosyl ceramide synthase, thapsigargin, β -lapachone, 3-substituted β -lapachones, dunnione, ceramide, calcium iontophore, and 4-substituted-1,2-
15 naphthoquinones can be prepared using generally known reactions, techniques and synthetic procedures available to those of ordinary skill in the art. Several suitable syntheses for the preparation of 4-substituted-1,2-naphthoquinones have been described in
20 the art.

 Various reactions may be performed in solvents which are appropriate to the reagents and materials employed therein, and which are suitable for the transformation or syntheses being effected. It is
25 understood by those skilled in the art of organic synthesis that a functionality present on portions of a given molecule must be compatible with the reagents and reaction conditions used.

 Although the present invention is broadly
30 applicable to delivery of any pro-apoptotic agent to the prostate, the invention is expressly exemplified herein with respect to the synthesis of the following preferred pro-apoptotic compounds: 4-substituted-1,2-naphthoquinones, including 4-alkoxy-1,2-naphthoquinone,
35 4-penthanethio-1,2-naphthoquinone, and 4-pentylamino-1,2-naphthoquinone.

Synthesis of 4-substituted-1,2-naphthoquinones.

Synthesis of 4-substituted-1,2-naphthoquinones can be accomplished using standard methodologies. The starting materials, 1,2-naphthoquinone and 2-hydroxy-1,4-naphthoquinone (lawsone), and all required reagents, are widely available from several international commercial suppliers, including the Sigma Chemical Company (St. Louis, Missouri, USA).

10

Preparation of 4-alkoxy-1,2-naphthoquinones.

The synthesis of 4-alkoxy-1,2-naphthoquinones can be accomplished using methods known in the art. For example, synthesis can proceed via the silver salt of 2-hydroxy-1,4-naphthoquinone (lawsone). An illustrative synthesis, showing the preparation of 4-isopropoxy-1,2-naphthoquinone, proceeds as follows. To 750 mL of hot water is added 50.0 g (0.287 mol) of 2-hydroxy-1,4-naphthoquinone. Sufficient ammonium hydroxide is added to bring the quinone into solution. Then 287 mL of 1 M AgNO₃ is added, and the mixture cooled in an ice-bath. The red salt is collected by filtration and washed successively with water, ethanol, and diethyl ether and dried. Yields using this method are generally 76.13 g (94%) of silver salt.

To a suspension of 5.00 g (17.8 mM) of the silver salt of 2-hydroxy-1,4-naphthoquinone in 50 mL of benzene is added 1.85 mL (19.7 mM) of 2-bromopropane. The reaction mixture is heated at 50°C for 24 hr, and the benzene removed under reduced pressure. The crude material is treated with ethyl acetate to dissolve the organic products, and the resultant solution filtered to remove silver salts. The ethyl acetate solution is extracted first with cold 10% NH₄OH until the extract is colorless, and then with 5% NaHSO₃ until the extract gives no

precipitate of orthoquinone upon addition of Na_2CO_3 solution. The NaHSO_3 extracts are combined, treated with Na_2CO_3 solution, and extracted with CH_2Cl_2 . The CH_2Cl_2 extracts are combined and dried with MgSO_4 .

5 Removal of solvent yields approximately 2 g (53%) of 4-(isopropoxy-1,2-naphthoquinone, which can be crystallized from benzene-ligroin. The properties of the synthesized compound are generally as follows: mp 125--125.5°C; ^1H NMR (300 MHz, CDCl_3 , TMS) δ 8.12 (dd, J = 8.1 Hz, 1H), 7.89 (dd, J = 8.1 Hz, 1H), 7.69 (td, J = 8.1 Hz, 1H), 7.59 (td, J = 8.1 Hz, 1H), 5.96 (s, 1H), 4.75 (septet, J = 6 Hz, 1H), 1.49 (d, J = 6 Hz, 6H); ^{13}C NMR (75.5 MHz, CDCl_3 , TMS) δ 179.5, 179.3, 166.7, 134.8, 132.3, 131.3, 130.4, 128.6, 124.8, 103.7, 72.9,

10 = 8.1 Hz, 1H), 7.89 (dd, J = 8.1 Hz, 1H), 7.69 (td, J = 8.1 Hz, 1H), 7.59 (td, J = 8.1 Hz, 1H), 5.96 (s, 1H), 4.75 (septet, J = 6 Hz, 1H), 1.49 (d, J = 6 Hz, 6H); ^{13}C NMR (75.5 MHz, CDCl_3 , TMS) δ 179.5, 179.3, 166.7, 134.8, 132.3, 131.3, 130.4, 128.6, 124.8, 103.7, 72.9,

15 21.4. Analysis calculated for $\text{C}_{13}\text{H}_{12}\text{O}_3$: C = 72.20, H = 5.60; Found: C = 72.06, H = 5.62.

The following 4-alkoxy-1,2-naphthoquinones, prepared as described above, are particularly preferred for use in the methods of the invention: 4-pentoxo-1,2-naphthoquinone (MW = 244.31); 4-cyclohexymethoxy-1,2-naphthoquinone (MW = 270.35); and bis-6[(1,2-naphthoquinone-4-yl)oxy]hexyloxy carbonate (MW = 574). These compounds are generally soluble in DMSO-saline, methanol, and organic solvents.

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Preparation 4-thio-1,2-naphthoquinones.

An illustrative synthesis for the preparation of 4-pentanethio-1,2-naphthoquinone, proceeds as follows. To a solution of 1.00 g (6.32 mM) of 1,2-naphthoquinone in 150 mL of methanol is added 784 μL (6.32 mM) of pentanethiol. The reaction mixture is stirred at room temperature for 22 hr. Removal of the methanol and column chromatography on silica gel with CH_2Cl_2 affords approximately 100 mg (6%) of 4-pentanethio-1,2-naphthoquinone, which can be recrystallized from hexanes. The properties of the synthesized compound are as follows: mp 107-108°C; ^1H

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NMR (300 MHz, CDCl_3 , TMS) δ 8.17 (d, $J = 7.7$ Hz, 1H), 7.85 (d, $J = 7.7$ Hz, 1H), 7.68 (t, $J = 7.7$ Hz, 1H), 7.57 (t, $J = 7.7$ Hz, 1H), 6.42 (s, 1H), 3.04 (t, $J = 7$ Hz, 2H), 1.9-1.8 (m, 2H), 1.5-1.3 (m, 4H), 0.95 (t, $J = 7$ Hz, 3H); ^{13}C NMR (75.5 MHz, CDCl_3 , TMS) δ 179.6, 176.2, 160.2, 134.9, 133.7, 131.1, 130.4, 129.2, 125.2, 119.7, 31.8, 31.1, 27.2, 22.2, 13.8. Analysis calculated for $\text{C}_{15}\text{H}_{16}\text{O}_2\text{S}$: C = 69.19, H = 6.21, S = 12.31; Found C = 68.97, H = 6.22, S = 12.31.

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Preparation of 4-amino-1,2-naphthoquinones.

The amino derivatives can be obtained via reaction of the alkoxy derivatives with an appropriate amine. An illustrative synthesis of 4-pentylamino-1,2-naphthoquinone proceeds as follows. To a stirred suspension of 650 mg (3.45 mM) of 4-methoxy-1,2-naphthoquinone in 20 mL of ethanol is added 0.80 mL (6.90 mM) of pentylamine. The reaction mixture is stirred at room temperature for 60 min, the resulting red precipitate collected by filtration, and then triturated with 20 mL of ethyl acetate to afford approximately 400 mg (49%) of 4-pentylamino-1,2-naphthoquinone, which can be recrystallized from ethyl acetate. The properties of the synthesized compound are as follows: mp $>215^\circ\text{C}$; ^1H NMR (300 MHz, CDCl_3 , TMS) δ 8.21 (dd, $J = 7.5, 1.6$ Hz, 1H), 7.69 (td, $J = 7.7, 1.6$ Hz, 1H), 7.61 (t, $J = 7.5$ Hz, 1H), 7.49 (d, $J = 7.9$ Hz, 1H), 5.86 (s, 1H), 5.76 (br s, 1H), 3.4-3.3 (m, 2H), 1.8-1.7 (m, 4H), 1.5-1.4 (m, 4H), 0.95 (t, $J = 7$ Hz, 3H); ^{13}C NMR (75.5 MHz, d_6 -DMSO, TMS) δ 182.0, 174.7, 154.8, 134.1, 131.2, 130.8, 127.8, 123.3, 98.0, 43.1, 28.7, 27.4, 21.8, 13.9. Analysis calculated for $\text{C}_{15}\text{H}_{17}\text{NO}_2$: C = 74.04, H = 7.06, N = 5.7; Found: C = 73.88, H = 7.01, N = 5.73.

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Once prepared, the pro-apoptotic compound can be combined with a pharmaceutically acceptable

carrier, and/or other appropriate materials, to formulate a suitable pharmaceutical composition. Some appropriate materials used to formulate pharmaceutical compositions used herein include buffers, excipients, gels, matrices and polymers known in the drug delivery arts. The pharmaceutical compositions may also contain auxiliary substances such as emulsifying, preserving or wetting agents, or the like.

Alternative formulations include liposomal preparations such as, for example, those disclosed in U.S. Patent No. 5,422,120, International Publication Nos. WO 95/13796, WO 94/23697, WO 91/14445, and in European Application EP 0 524 968-B1. Particularly preferred formulations comprise heterovesicular liposomal preparations. Liposome formulations can provide increased and/or sustained delivery of pharmaceuticals, and are thus particularly well-suited to the methods of the invention.

(B). Preparation of Pro-Apoptotic Compositions for Gene Therapy

The following material details a number of promoters and vectors useful for gene therapy applications of the invention, particularly, for prostatic delivery of polynucleotides in plasmids, viral vectors or liposomes. Although the methodology described below is believed to contain sufficient details to enable one skilled in the art to practice the present invention, other items not specifically exemplified, such as plasmids, can be constructed and purified using standard recombinant DNA techniques described in, for example, Sambrook et al. (1989) *Molecular Cloning, a Laboratory Manual*, 2d edition (Cold Spring Harbor Press, Cold Spring Harbor, N.Y.), and Ausubel et al. (1994) *Current Protocols in Molecular Biology*, (Greene Publishing Associates and John Wiley & Sons, New York, N.Y.), and in the current

regulations described in United States Dept. of HHS, National Institute of Health (NIH) Guidelines for Recombinant DNA Research. These references include procedures for the following standard methods: cloning
5 procedures with plasmids, transformation of host cells, cell culture, plasmid DNA purification, phenol extraction of DNA, ethanol precipitation of DNA, agarose gel electrophoresis, purification of DNA fragments from agarose gels, and restriction
10 endonuclease and other DNA-modifying enzyme reactions.

The coding sequences of pro-apoptotic agents are generally known in the art. For example, coding sequences for fas (Itoh et al. (1991) *Cell* 66:233-243, Nagata (1994) *Adv. in Immunol.* 57:129-144), fas ligand
15 (European Application No. EP 675,200, Suda et al. (1993) *Cell* 75:1169-1178, Takahashi et al. (1994) *Int'l Immunol.* 6:1567-1574, Nagata (1994) *Adv. in Immunol.* 57:129-144, Tanaka et al. (1995) *EMBO J.* 14:1129-1135), fadd (Kim et al. (1996) *J. Immunol.* 157:5461-5466), fibroblast activation protein-1 ("fap-1") (Scanlan et al. (1994) *Proc. Natl. Acad. Sci USA* 91:5657-5661), tradd (Pan et al. (1995-1996) *J. of Inflammation* 46:168-175), fibroblast activating factor ("faf") (Mihara et al. (1993) *Infect. and Immunity* 61:588-595), ribosome inactivating protein ("rip")
25 (Parente et al. (1993) *Biochem Biophys. Acta* 1216:43-49), reaper, apoptin (Zuang et al. (1995) *Leukemia* 9 Supp (1):5118-5120), interleukin-2 converting enzyme (ICE) (Keane et al. (1995) *Cytokine* 7:105-110, Pai et al. (1996) *Proc. Natl. Acad. Sci. USA* 93:5437-5442),
30 FADD-homologous ICE-CED3-like protease (FLICE) (Muzio et al. (1996) *Cell* 85:817-827), bax (Apte et al. (1995) *Genomics* 26:592-594, Bargon et al. (1996) *J. of Clin. Inv.* 97:2651-2659), P53 (Miyashita et al. (1995) *Cell* 80:293-299, Gjerset et al. (1995) *Molecular Carcinogenesis* 14:275-285), TNF receptor type-I (Santee et al. (1996) *J. Biol. Chem.* 271:21151-21159),

TNF-alpha (Gillio et al. (1996) Blood 87:2486-2495), and cytochrome-c, can be readily obtained, and either used directly, or inserted into plasmids for expression of a pro-apoptotic agent *in vivo* or *ex vivo*.

More particularly, isolated coding regions for pro-apoptotic agents can be operably linked to control elements that direct the transcription or expression thereof using standard ligation techniques, such as those described in Sambrook et al., *supra*. For example, ligations can be accomplished in 20 mM Tris-Cl pH 7.5, 10 mM MgCl₂, 10 mM DTT, 33 µg/ml BSA, 10 mM-50 mM NaCl, and either 40 µM ATP, 0.01-0.02 (Weiss) units T4 DNA ligase at 0°C (for "sticky end" ligation) or 1 mM ATP, 0.3-0.6 (Weiss) units T4 DNA ligase at 14°C (for "blunt end" ligation). Intermolecular "sticky end" ligations are usually performed at 30-100 µg/ml total DNA concentrations (5-100 nM total end concentration).

Alternatively, synthetic methods can be used to produce polynucleotide molecules including coding sequences for a pro-apoptotic agent, alternatively linked to suitable control elements. The complete sequence can be assembled from overlapping oligonucleotides prepared by standard methods. See, e.g., Edge, *Nature* (1981) 292:756; Nambair et al. *Science* (1984) 223:1299; Jay et al. *J. Biol. Chem.* (1984) 259:6311.

Control elements can comprise control sequences normally associated with the selected coding sequence. Alternatively, heterologous control sequences can be employed. Useful heterologous control sequences generally include those derived from sequences encoding mammalian or viral genes. Examples include, but are not limited to, the SV40 early promoter, adenovirus major late promoter (Ad MLP), a herpes simplex virus (HSV) promoter, a cytomegalovirus

(CMV) promoter such as the CMV immediate early promoter region (CMVIE), a rous sarcoma virus (RSV) promoter, synthetic promoters, hybrid promoters, and the like.

5 If desired, the coding sequence may be linked to tissue specific promoters or leader sequences for expression in prostate cells, for example, promoters of prostate specific antigen (PSA), kallikrein-1, prostate specific membrane antigen
10 (PSMA), and prostatic alkaline phosphatase (PAP).

 Expression of the coding sequence *in vivo* can be regulated for maximal efficacy and safety through the use of regulated gene expression promoters as described in Gossen et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:5547-5551. For example, a selected coding
15 sequence can be operably linked to a tetracycline responsive promoter or other inducible promoter. These promoters can be regulated in a positive or negative fashion by treatment with the appropriate
20 regulator molecule.

 Once prepared, the above-described polynucleotide molecules can be used for treatment of prostatic disorders by gene therapy. Delivery of the polynucleotides can be carried out via viral or non-
25 viral vector approaches, and can be practiced either *in vivo* or *ex vivo*.

 For delivery using viral vectors, any of a number of viral vectors conventional in the art can be used. For example, the coding sequence can be
30 inserted into plasmids designed for expression in retroviral vectors, as described in Kimura et al. (1994) *Human Gene Therapy* 5:845-852, adenoviral vectors, as described in Connelly et al. (1995) *Human Gene Therapy* 6:185-193, or adeno-associated viral
35 vectors, as described in Kaplitt et al. (1994) *Nature Genetics* 6:148-153. The vector can also be an astrovirus, herpesvirus, alphavirus, coronavirus,

orthomyxovirus, papovavirus, paramyxovirus, parvovirus, picornavirus, poxvirus, sinbus, or togavirus viral vector. Replication-incompetent viral particles can be produced and injected directly into the subject, or introduced to the subject by transduction of an autologous cell *ex vivo*, followed by injection *in vivo* as described in Zatloukal et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:5148-5152.

Gene delivery is not limited to the above mentioned viral vectors. Other delivery methods and media may be employed such as, for example, nucleic acid expression vectors, polycationic condensed DNA linked or unlinked to killed adenovirus alone (see Curiel (1992) *Hum Gene Ther* 3:147-154), ligand linked DNA (Wu (1989) *J. Biol Chem* 264:16985-16987), eucaryotic cell delivery vehicle cells (see commonly-owned U.S. Application Serial Nos. 08/240,030 and 08/404,796), deposition of photopolymerized hydrogel materials, gene transfer particle guns (U.S. Patent No. 5,149,655), ionizing radiation (U.S. Patent No. 5,206,152, International Publication No. WO 92/11033), nucleic charge neutralization, or fusion with cell membranes. Additional approaches are described in Philip (1994) *Mol Cell Biol* 14:2411-2418, and in Woffendin (1994) *Proc Natl Acad Sci USA* 91:1581-585.

Particle mediated gene transfer may also be employed. Briefly, the coding sequence for the pro-apoptotic agent can be inserted into vectors that contain conventional control sequences for high level expression. The resultant vectors can then be incubated with synthetic gene transfer molecules (e.g., with polymeric DNA-binding cations like polylysine, protamine, and albumin), or linked to cell targeting ligands such as asialoorosomucoid (Wu et al. (1987) *J. Biol. Chem.* 262:4429-4432), insulin (Hucked (1990) *Biochem Pharmacol* 40:253-263), galactose (Plank

(1992) *Bioconjugate Chem* 3:533-539), lactose, and transferrin.

Naked DNA delivery techniques may also be employed. Exemplary naked DNA introduction methods are described in International Publication No. WO 90/11092 and U.S. Patent No. 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA-coated latex beads are efficiently transported into cells by endocytosis. The method may be improved further by treating the beads to increase hydrophobicity, thereby facilitating disruption of the endosome and release of DNA into the cytoplasm.

Any of the above coding sequences, polynucleotide molecules, or vectors, can be combined with a buffer or other pharmaceutically acceptable carrier to provide a pro-apoptotic pharmaceutical composition. The selected carrier can serve to stabilize the nucleic acids, facilitate transduction thereof into target cells, and/or provide targeting as described in Zhu et al. (1993) *Science* 261:209-211. Furthermore, liposomal formulations can be prepared. Liposomes suitable for gene delivery vehicles are described in U.S. Patent No. 5,422,120, in International Publication Nos. WO 95/13796, WO 94/23697, WO 91/144445, and in European Patent Application No. EP 524,968.

Other delivery systems include the use of liposomes to encapsulate a pro-apoptotic sequence under the control of a variety of tissue-specific or ubiquitously-active promoters. Exemplary liposome and polycationic gene delivery vehicles include those described in U.S. Patent Nos. 5,422,120 and 4,762,915; International Publication Nos. WO 95/13796, WO 94/23697, and WO 91/14445; European Application No. EP 0524968; Stryer (1975) *Biochemistry*, pages 236-240, W.H. Freeman, San Francisco, CA; Szoka (1980) *Biochem Biophys Acta* 600:1; Bayer (1979) *Biochem Biophys Acta*

550:464; Rivnay (1987) *Meth Enzymol* 149:119; Wang (1987) *Proc Natl Acad Sci USA* 84:7851; and Plant (1989) *Anal Biochem* 176:420.

5 (C). Preparation of Polypeptide Pro-Apoptotic Compositions

 The above-described polypeptide pro-apoptotic agents can be prepared using known techniques. See Sambrook et al., *supra*. More particularly, pro-apoptotic polypeptides, (and alleles, variants and functionally active fragments thereof) can be expressed using any suitable expression system, including, for example, bacterial, yeast, insect, amphibian and mammalian systems.

10 Expression systems in bacteria include those described in Chang et al. (1978) *Nature* 275:615, Goeddel et al. (1979) *Nature* 281:544, Goeddel et al. (1980) *Nucleic Acids Res.* 8:4057, European Application No. EP 36,776, U.S. Patent No. 4,551,433, deBoer et al. (1983) *Proc.*

15 *Natl. Acad. Sci. USA* 80:21-25, and Siebenlist et al. (1980) *Cell* 20:269.

 Expression systems in yeast include those described in Hinnen et al. (1978) *Proc. Natl. Acad. Sci. USA* 75:1929, Ito et al. (1983) *J. Bacteriol.*

25 153:163, Kurtz et al. (1986) *Mol. Cell. Biol.* 6:142, Kunze et al. (1985) *J. Basic Microbiol.* 25:141, Gleeson et al. (1986) *J. Gen. Microbiol.* 132:3459, Roggenkamp et al. (1986) *Mol. Gen. Genet.* 202:302, Das et al. (1984) *J. Bacteriol.* 158:1165, De Louvencourt et al. (1983) *J. Bacteriol.* 154:737, Van den Berg et al. (1990) *Bio/Technology* 8:135, Kunze et al. (1985) *J. Basic Microbiol.* 25:141, Cregg et al. (1985) *Mol. Cell. Biol.* 5:3376, U.S. Patent Nos. 4,837,148 and 4,929,555, Beach et al. (1981) *Nature* 300:706, Davidow et al. (1985) *Curr. Genet.* 10:380, Gaillardin et al.

30 (1985) *Curr. Genet.* 10:49, Ballance et al. (1983) *Biochem. Biophys. Res. Commun.* 112:284-289, Tilburn et

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al. (1983) *Gene* 26:205-221, Yelton et al. (1984) *Proc. Natl. Acad. Sci. USA* 81:1470-1474, Kelly et al. (1985) *EMBO J.* 4:475479; European Application No. EP 244,234, and International Publication No. WO 91/00357.

- 5 Expression of heterologous genes in insects can be accomplished as described in U.S. Patent No. 4,745,051, Friesen et al. (1986) *The Regulation of Baculovirus Gene Expression*, in: *THE MOLECULAR BIOLOGY OF BACULOVIRUSES* (W. Doerfler, ed.), European
- 10 Application Nos. EP 127,839 and EP 155,476, Vlak et al. (1988) *J. Gen. Virol.* 69:765-776, Miller et al. (1988) *Ann. Rev. Microbiol.* 42:177, Carbonell et al. (1988) *Gene* 73:409, Maeda et al. (1985) *Nature* 315:592-594, Lebacqz-Verheyden et al. (1988) *Mol. Cell.*
- 15 *Biol.* 8:3129, Smith et al. (1985) *Proc. Natl. Acad. Sci. USA* 82:8404, Miyajima et al. (1987) *Gene* 58:273, and Martin et al. (1988) *DNA* 7:99. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts are described
- 20 in Luckow et al. (1988) *Bio/Technology* 6:47-55, Miller et al. (1986) *GENERIC ENGINEERING*, Setlow, J.K. et al. eds., Vol. 8, Plenum Publishing, pp. 277-279, and Maeda et al. (1985) *Nature* 315:592-594.

- Mammalian expression can be accomplished as
- 25 described in Dijkema et al. (1985) *EMBO J.* 4:761, Gorman et al. (1982) *Proc. Natl. Acad. Sci. USA* 79:6777, Boshart et al. (1985) *Cell* 41:521, and U.S. Patent No. 4,399,216. Other features of mammalian expression can be facilitated as described in Ham et
- 30 al. (1979) *Meth. Enz.* 58:44, Barnes et al. (1980) *Anal. Biochem.* 102:255, U.S. Patent Nos. 4,767,704, 4,657,866, 4,927,762, 4,560,655 and Reissued U.S. Patent No. RE 30,985, and in International Publication Nos. WO 90/103430, WO 87/00195.

- 35 Once expressed, fas (Itoh et al. (1991) *Cell* 66:233-243, Nagata (1994) *Adv. in Immunol.* 57:129-144), fas ligand (European Application No. EP 675,200,

- Suda et al. (1993) *Cell* 75:1169-1178, Takahashi et al. (1994) *Int'l Immunol.* 6:1567-1574, Nagata (1994) *Adv. in Immunol.* 57:129-144, Tanaka et al. (1995) *EMBO J.* 14:1129-1135), fadd (Kim et al. (1996) *J. Immunol.* 157:5461-5466), fibroblast activation protein-1 ("fap-1") (Scanlan et al. (1994) *Proc. Natl. Acad. Sci USA* 91:5657-5661), tradd (Pan et al. (1995-1996) *J. of Inflammation* 46:168-175), fibroblast activating factor ("faf") (Mihara et al. (1993) *Infect. and Immunity* 61:588-595), ribosome inactivating protein ("rip") (Parente et al. (1993) *Biochem Biophys. Acta* 1216:43-49), reaper, apoptin (Zuang et al. (1995) *Leukemia* 9 Supp (1):5118-5120), interleukin-2 converting enzyme (ICE) (Keane et al. (1995) *Cytokine* 7:105-110, Pai et al. (1996) *Proc. Natl. Acad. Sci. USA* 93:5437-5442), FADD-homologous ICE-CED3-like protease (FLICE) (Muzio et al. (1996) *Cell* 85:817-827), bax (Apte et al. (1995) *Genomics* 26:592-594, Bargon et al. (1996) *J. of Clin. Inv.* 97:2651-2659), P53 (Miyashita et al. (1995) *Cell* 80:293-299, Gjerset et al. (1995) *Molecular Carcinogenesis* 14:275-285), TNF receptor type-I (Santee et al. (1996) *J. Biol. Chem.* 271:21151-21159), TNF-alpha (Gillio et al. (1996) *Blood* 87:2486-2495), and cytochrome-c can be combined with one or more "pharmaceutically acceptable excipients or vehicles" such as water, saline, glycerol, ethanol, etc., to provide a pharmaceutical composition. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in the composition.

(D). Preparation of Anti-Proliferative Compounds and Compositions

- A number of suitable anti-proliferative compounds and compositions can be readily prepared for use with the present invention. For example, daunorubicin, dactinomycin, doxorubicin, methotrexate,

etoposide, estramustine, cyclophosphamide, cisplatin, suramin, and polyamine analogs possessing an additional subunit of $(CH_2)_nNH_2$ relative to spermine and N1, N14-bis(ethyl)-amino-5, 10-diazatetradecane (BE-4-4-4), can be prepared using generally known reactions, techniques and synthetic procedures available to those of ordinary skill in the art. The synthesis of suitable polyamine analogs for use herein is described in U.S. Patent No. 5,541,230.

Various reactions may be performed in solvents which are appropriate to the reagents and materials employed therein, and which are suitable for the transformation or syntheses being effected. It is understood by those skilled in the art of organic synthesis that a functionality present on portions of a given molecule must be compatible with the reagents and reaction conditions used.

Although the present invention is broadly applicable to delivery of any anti-proliferative agent to the prostate, the invention is expressly exemplified herein with respect to the synthesis of the following preferred anti-proliferative compounds: polyamine analogs including N¹, N¹⁹-bis-(ethylamino)-5,10,15-triazanonadecane ("BE4x4").

25

Synthesis of polyamine analogs

Polyamine analogs that possess an additional subunit of $(CH_2)_nNH_2$ relative to spermine and N1, N14-bis(ethyl)-amino-5, 10-diazatetradecane (BE-4-4-4) have been shown to retain the ability to enter and disable cellular growth pathways. Polyamine analogs used in the methods of the present invention have the general formula:

(I) $R_1-NH-(CH_2)_w-NH-(CH_2)_x-NH-(CH_2)_y-NH-(CH_2)_z-NH-R_2$,
wherein R_1 and R_2 are hydrocarbon chains having 1 to 5 carbons and w , x , y and z are integers of 1 to 10. Preferably, R_1 and R_2 are hydrocarbon

chains having 2 to 4 carbons, and w, x y and z are integers of 2 to 5. Even more preferably, R₁ and R₂ are hydrocarbon chains having 2 carbons and w, x y and z are integers of 3 to 4.

5 Particularly preferred compounds have four subunits of (CH₂)_nNH₂, wherein n is an integer of 1 to 10. The presence of the four subunits appears to eliminate growth-promoting conformational activity in these compounds, as well as increasing their affinity for DNA as compared to the affinity of spermine. Additionally, groups R₁ and R₂ protect the compounds against degradation *in vivo*, and appear to add to the toxicity of the compounds.

 One preferred compound, N¹, N¹⁹-bis-
15 (ethylamino)-5,10,15-triazanonadecane ("BE4x4"), binds DNA better than spermine, but does not impact the conformational changes to DNA which are caused by spermine. By binding to DNA, but not effecting growth-promoting conformational changes, BE4x4
20 displaces the natural polyamines and prevents the conformational changes required for growth.

Synthesis of BE4x4.

 A variety of known chemical reactions can be
25 employed to synthesize BE4x4. The synthesis of BE4x4 is described in U.S. Patent No. 5,541,230. One approach is to use two intermediates, namely N-p-toluenesulfonyl-N-ethyl-4-bromobutylamine and N¹,N⁵,N⁹-tribenzyl-5-aza-1,9-diaminononane.

30 To synthesize the first intermediate, N-p-toluenesulfonyl-N-ethyl-4-bromobutylamine, 18.4 mM EtNH₂HCl, 6 mL Et₂NH and 18.8 mM tosyl chloride are dissolved in 60 mL CH₂Cl₂, and the mixture is stirred for 12 hours at room temperature. About 10 mL H₂O is
35 added and stirring is continued for another two hours in order to decompose excess tosyl chloride. After washing with water and filtering with paper, the

organic phase is evaporated. The residue (tosylated ethylamine) is then dissolved in methyl alcohol and recrystallized.

Next, 12.6 mM tosylated ethylamine is
5 dissolved in 36 mL dry N,N-dimethylformamide ("DMF").
Thereafter, 17 mM NaH (60% in oil) is added to the
solution at room temperature. Hydrogen gas evolves
and the reaction mixture becomes sticky. Thirty
10 minutes after adding NaH, 73.7 mM 1,4-dibromobutane is
added to the sticky solution. after vigorous
stirring, the solution becomes clear. The solution is
then slowly stirred overnight. The reaction mixture
is then added to 200 mL of cold water and thereafter
15 extracted twice with 200 mL ether. The ether phase is
washed with water and then evaporated. The resulting
oily residue is dissolved in benzene, which in turn is
placed on a 120 g silica gel column equilibrated in
benzene. After eluting with 500 mL benzene, the
20 solvent is changed to a mixture of benzene and acetone
(20:1). Fractions are collected and evaporated to
obtain pure oily N-p-toluenesulfonyl-N-ethyl-4-
bromobutylamine.

To synthesize the second intermediate,
N¹,N⁵,N⁹-tribenzyl-5-aza-1,9-diaminononane, N-(4-
25 bromobutyl)phthalimide is first synthesized from a
mixture of 30 mM potassium phthalimide and 100 mM 1,4
dibromobutane in 50 mL DMF. The solution is stirred
for 2 hours at 60°C, and the DMF and 1,4-dibromobutane
are evaporated off in vacuo. The remaining residue is
30 extracted with CHCl₃ and H₂O. The resulting white
compound is recrystallized from ethyl alcohol. See
Chem. Pharm. Bull, 32:3428 (1984).

A stirred solution of 6 mM benzylamine and
14 mM N-(4-bromobutyl)phthalimide in 24 mL CH₃CN was
35 refluxed for 14 hours in the presence of 2.4 g KF-
Celite. After removal of the KF-Celite, the filtrate
is evaporated in vacuo and the residue is dissolved in

10 mL benzene. This solution is then placed on a 20 g silica gel column equilibrated with benzene. The column is then successively eluted with 100 ml benzene and 100 ml benzene/acetone (20:1). The solvent is
5 then removed from the last fraction. See *Chem. Pharm. Bull.* 34:1032-1038 (1986).

A solution of N,N-bis(4-phthalimido-butyl)benzylamine (3.7 mM) in 34 mL methyl alcohol containing 2.8 mL $\text{NH}_2\text{NH}_2\text{H}_2\text{O}$ is refluxed for 3 hours and
10 then evaporated in vacuo. The residue is shaken for 30 minutes with 34 mL of CHCl_3 and 34 mL 4N NH_4OH . The ammonia phase is re-extracted with 34 mL CHCl_3 . The CHCl_3 fractions are combined, filtered with paper and evaporated. This yields 3.5 mM N^5 -benzyl-5-aza-1,9-
15 diaminononane, which is dissolved in 14 mL methyl alcohol containing 7.0 mM benzaldehyde and 1.1 g MgSO_4 . The solution is stirred for one hour at room temperature. Then, the solution is placed in an ice bath, and 0.8 g NaBH_4 and 10 mL methyl alcohol is
20 added to the solution over a period of 1 hour. The solution is then stirred for an additional hour. The methyl alcohol is then removed in vacuo, and the residue is extracted twice with 25 mL Et_2O and once with 25 mL H_2O . The Et_2O extracts are combined and
25 then washed twice with 25 mL of H_2O . The Et_2O is then removed, yielding $\text{N}^1, \text{N}^5, \text{N}^9$ -tribenzyl-5-aza-1,9-diaminonona.

To synthesize BE4x4 using the above-described intermediates, 35.5 mM N-p-toluenesulfonyl-
30 N-ethyl-4-bromobutylamine and 14.8 mM $\text{N}^1, \text{N}^5, \text{N}^9$ -tribenzyl-5-aza-1,9-diaminononane are dissolved in 60 mL CH_3CN , which is then stirred and refluxed in the presence of 15.3 g KF celite for 16-18 hours. The reaction mixture is filtered and the filtrate is
35 evaporated to dryness. The residue is then dissolved in benzene, loaded on a silica gel column (300 g), and eluted with benzene/acetone (5:1). The purified

product is hydrolyzed in 6M HCl at 120°C for 5 days, followed by reductive elimination of benzyl groups with 10% palladium/carbon in a hydrogen atmosphere. The BE4x4 obtained from this process is crystallized from aqueous ethyl alcohol as a pentahydrochloride salt.

Once prepared, the anti-proliferative compound can be combined with a pharmaceutically acceptable carrier, and/or other appropriate materials, to formulate a suitable pharmaceutical composition. Some appropriate materials used to formulate pharmaceutical compositions used herein include buffers, excipients, gels, matrices and polymers known in the drug delivery arts.

Alternative formulations include liposomal preparations such as, for example, those disclosed in U.S. Patent No. 5,422,120, International Publication Nos. WO 95/13796, WO 94/23697, WO 91/14445, and in European Application EP 0 524 968-B1. Particularly preferred formulations comprise heterovesicular liposomal preparations. Liposome formulations can provide increased and/or sustained delivery of pharmaceuticals, and are thus particularly well-suited to the methods of the invention.

25

(E). Delivery of Pro-Apoptotic and/or Anti-Proliferative Compounds and Compositions

Once prepared, the above-described compounds and compositions can be administered to the prostate gland, or delivered to regions near the prostate gland in such a fashion that the pharmaceutical agents localize in the prostate. For example, in humans, the prostate surrounds the urethra, and resides near the bladder. Therefore, one method of administration entails administering the agent into the urethra to a position adjacent to the prostate. In this manner, the pharmaceutical agent can pass to the prostate

gland through the wall of the urethra, or pass to the prostate via the prostatic ducts.

Another technique to administer a pharmaceutical agent to the prostate entails delivery of the agent into the periprostatic space, i.e., the space immediately surrounding the prostate which can include soft tissue and lymphatic ducts adjacent to the prostate gland. The targeted area may be the entire region comprising the periprostatic space, or only a part of it. Thus, as used herein, administration of an agent into periprostatic space includes delivery to subregions of the periprostatic space that form interfaces between the periprostatic space, and the tissue that surrounds and forms it, for example soft tissue and lymphatic ducts surrounding the prostate gland.

Administration into periprostatic space or to the prostate can be accomplished by initially delivering the agent into the urethra, and allowing the subsequent diffusion of particles for local delivery to prostatic and periprostatic tissue. Alternatively, transurethral delivery of the agent to the prostate can be assisted by pressure, heat, and/or phoresis; particularly, iontophoresis, electrophoresis, or phonophoresis. Iontophoretic, electrophoretic and phonophoretic devices are generally known and available, and have been described, for example, in U.S. Patent Nos. 4,411,648, 5,222,936, 5,232,441, and 5,286,254; and in International Publication No. WO 94/05369.

Yet another technique to administer a pharmaceutical agent to the prostate entails direct injection of a pharmaceutical composition into the prostate. When direct injection is used, the pharmaceutical compositions are prepared as injectables, e.g., liquid solutions or suspensions; or

as solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection.

Other techniques entail topical administration of the pharmaceutical agent onto the urethra. Methods for preparing various dosage forms suitable for topical administration are known, or will be apparent, to those skilled in this art; for example, see Remington's Pharmaceutical Sciences, 18th Ed. (Easton, PA: Mack Publishing Company, 1990). For example, ointments, creams and emulsions containing the pharmaceutical agent can be prepared using known techniques. A variety of suitable pharmaceutical ointment bases are generally known, including oleaginous bases, anhydrous absorption bases, and oil-in-water (o/w) bases.

Pharmaceutical emulsion formulations are generally formed from a dispersed phase (e.g., the pharmaceutically active agent), a dispersion medium and an emulsifying agent. If desired, emulsion stabilizers can be included in the formulation as well. A number of pharmaceutically useful emulsions are known in the art, including oil-in-water (o/w) formulations, water-in-oil (w/o) formulations and multiple emulsions such as w/o/w or o/w/o formulations. Emulsifying agents suitable for use in such formulations include, but are not limited to, Tween 60®, Span 80®, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate and sodium lauryl sulfate.

In some applications, it may be desired to use a topical delivery platform that is capable of providing a pulsatile, continuous, cyclical or diurnal agent release profile, such as platforms formed from bioerodible coating materials and/or bioerodible polymers, wherein the pharmaceutical agent is dispersed within a polymer matrix. Such polymers are selected such that they bioerode in the moisture

present in the urethra, and provide for sustained agent release at readily predictable rates.

Bioerodible coating materials may be selected from a variety of natural and synthetic materials, including
5 gelatins, carnauba wax, shellacs, ethylcellulose, cellulose acetate phthalate or cellulose acetate butyrate.

In one embodiment of the invention, a pro-apoptotic and/or anti-proliferative pharmaceutical
10 composition is transurethrally administered to a subject via a catheter. The method entails inserting a catheter into the urethra, positioning the catheter proximal to the prostate, and delivering a therapeutically effective amount of a pharmaceutical
15 composition into the urethra.

In a related embodiment, a method is provided for transurethral administration of a pharmaceutical agent using pressure-assisted catheter delivery. Typically, the amount of pressure used to
20 assist the delivery is in the range of about 10 to 50 inches of water. The exact amount of pressure used in different subjects can be determined by routine trials, and will fall within a broad range. Optimal pressures for a given subject will depend, in part, on
25 indicators such as physical size and overall health of the subject being treated. Application of pressure causes the pharmaceutical agent to be delivered either indirectly throughout the prostatic ducts into the prostate or periprostatic tissue, or directly through
30 the prostatic tissue itself. The invention is not limited, however, to any theories or mechanism of delivery.

Repeated or continuous administration over a period of time can be practiced in order to achieve
35 the goals of a particular therapy. Pressure-assisted catheter delivery can also be facilitated by administration of a chasing solution after the

pharmaceutical agent has been delivered into the urethra. The chasing solution can be a non-toxic solution such as saline or PEG, and is normally delivered from the catheter under pressure. Further,
5 heat can be combined with the above methods to further facilitate delivery of the agent to the prostate.

In another related embodiment, transurethral administration of a pharmaceutical agent to a subject's prostate is carried out using a combination
10 of catheter-based, and phoretic delivery techniques. In this regard, transurethral delivery of pharmaceutical agents to prostatic and/or periprostatic tissue can be achieved by transurethral catheterization to the prostate, combined with the
15 creation of an electric or ultrasonic field. The pharmaceutically active agents can thus be effectively pulse-chased past the urethra and into the hyperplastic or other prostatic tissue, achieving a localized delivery to the prostate gland while sparing
20 the urethra from exposure to the pro-apoptotic agents.

Phoretic-assisted catheter delivery can also be conducted by first using phoresis to deliver the agent, and then replacing the catheter with a second, preferably empty, catheter, and resuming phoresis to
25 push the delivered agent into the target tissue.

Phoretic devices used in such methods can contain electrically conductive elements and/or active electrodes to assist in iontophoresis or electrophoresis. Other materials and methods for
30 increasing the efficiency of electrodes are described in U.S. Patent Nos. 4,570,637, 4,747,819, and 5,222,936.

Application of an electric field to a pharmaceutical composition can help accelerate the
35 delivery rate of the composition into surrounding tissues. In addition, the use of pressure may be combined with phoresis to facilitate the delivery of

pharmaceutical agents to the prostate. The rate of delivery can be controlled by varying the strength of the electric field, which includes varying the current and/or the time the electric field is applied. The rate of delivery can also be adjusted by varying the amount of pressure applied.

The length of time that an electrical current is applied can of course be varied to provide a maximum transfer rate while avoiding potential problems caused by the electrical current. Typically, the electrical field is applied continuously for at least about 1 minute, more typically, at least about 5 minutes, and even more typically, about 15 minutes. However, the electrical field is usually not applied continuously for periods in excess of about 2 hours, more usually no more than about 1 hour, and even more usually, no more than about 30 minutes.

It will be understood that the time interval can be varied within and in the proximity of these ranges to maximize the rate of transfer for a given therapeutic agent or pharmaceutical composition. It is also understood that the optimal time interval and electrical field strength for delivery of a given therapeutic or mixture of therapeutics to a given subject diagnosed with a particular prostatic condition will vary depending on the nature of the pharmaceutical agent, the condition of the subject, and other parameters particular to the biological condition being treated. It is also contemplated by the invention that multiple administrations by phoretic-assisted catheter delivery can and may be conducted to achieve optimal results.

Phonophoresis, sometimes referred to as sonophoresis, can be used as an alternative to iontophoresis to help transport pharmaceutical agents or compositions to the targeted prostatic tissue. One suitable method of phonophoretic delivery is described

in International Publication No. WO 94/05361.

Phonophoresis uses ultrasonic or high frequency sound waves to transport agents or compositions to targeted tissue. In certain circumstances, phonophoresis can
5 be more effective than electrophoresis or
iontophoresis in transporting uncharged pharmaceutical agents or compositions.

In the present methods, phonophoresis may also be used in conjunction with the application of
10 pressure for delivery of pharmaceuticals to the prostate. Other factors for optimizing phonophoretic delivery include tissue temperature, tissue hyperemia and capillary permeability. When phonophoresis is used, the cathode electrode used with iontophoretic
15 devices is replaced by an ultrasonic piezoelectric transducer, for example, a barium titanate or lead zirconate titanate transducer element, which is connected to an external ultrasound source.

Other phoretic means of delivery can be
20 practiced with the present methods, including, for example, electrophoretic protocols wherein certain pharmaceuticals preferentially diffuse into the prostatic tissue in the presence of an electrical field.

In all of the above-described methods of the
25 invention, steps can be taken to maximize perfusion of the prostatic tissues. For example, pressure- and/or phoretic-assisted catheterization can be used to administer proangiogenic factors to the prostate prior
30 to delivery of the pharmaceutical agent.

Proangiogenic agents serve to increase vascularization of prostate and periprostate tissue. Alternatively, perfusion channels can be made in the prostate tissue using a laser.

35 To practice the methods of the invention, diagnosis of a prostatic disorder or potential prostatic disorder is made, and the appropriate

pharmaceutical agent or agents are determined on the basis of the diagnosis. Thus, the invention is useful in the prevention, amelioration and/or treatment of prostatic disorders.

5 Subsequent to treatment, monitoring of the prostatic condition can be carried out by biopsy of the prostate tissue, sonogram, magnetic resonance spectroscopy, and other tests standard in the art for diagnosis and monitoring of a patient with a prostatic
10 condition.

 The pharmaceutical compositions are administered to the subject to be treated in a manner compatible with the dosage formulation, and in an amount that will be prophylactically and/or
15 therapeutically effective. The amount of the composition to be delivered depends on both the prostatic condition and the subject to be treated. Thus, the exact amount necessary will vary depending on the age and general condition of the individual to
20 be treated, the severity of the condition being treated and the particular pro-apoptotic and/or anti-proliferative agent selected and its mode of administration, among other factors. An appropriate effective amount can be readily determined by one of
25 skill in the art. Thus, a "therapeutically effective amount" of a particular pro-apoptotic or anti-proliferative composition will be sufficient to bring about treatment or prevention of prostatic condition symptoms, and will fall in a relatively broad range
30 that can be determined through routine trials.

 In addition, the pharmaceutical compositions can be given in a single or multiple dose schedule. A multiple dose schedule is one in which a primary course of administration may entail 1-10 separate
35 doses, followed by other doses given at subsequent time intervals as needed to maintain or reinforce the action of the compositions. Thus, the dosage regimen

will also, at least in part, be determined based on the particular needs of the subject to be treated and will be dependent upon the judgement of the reasonably skilled practitioner.

5 When the pharmaceutical compositions comprise 4-substituted-1,2-napthoquinones, a unit dosage will preferably provide a concentration of the agent at the point of contact with the targeted prostatic cell of from about 1 μ M to about 10 μ M, and
10 more preferably from about 1 μ M to about 100 μ M.

 When polyamine analogs are used, particularly BE4x4, therapeutically effective dosages will preferably be in the range of about 1-10 mg BE4x4/kg body weight b.i.d, preferably in the range of
15 about 5-6 mg BE4x4/kg body weight b.i.d. Typically, several treatment cycles using the above dosages will be effective.

 For polypeptide pro-apoptotic agents, therapeutically effective dosages will generally fall
20 within the following ranges: about 5 μ g to about 50 μ g/kg body weight; about 50 μ g to about 5 mg/kg body weight; about 100 μ g to about 500 μ g/kg body weight; and about 200 to about 250 μ g/kg body weight.

 For polynucleotide pharmaceutical
25 compositions, for example those used in gene therapy, coding sequences, or vectors including coding sequences, will be administered in the following ranges: about 100 ng to about 200 mg of DNA or RNA; about 500 ng to about 50 mg of DNA or RNA; about 1 μ g
30 to about 2 mg of DNA or RNA; about 5 μ g to about 500 μ g of DNA or RNA; and about 20 μ g to about 100 μ g DNA or RNA (for local administration in a gene therapy protocol), and, for example, a dosage of about 500 μ g
35 DNA or RNA (for systemic delivery via injection or other parenteral administration).

 Factors such as method of action and efficacy of transformation and expression are

considerations that will affect the dosage required for ultimate efficacy for DNA and nucleic acid pharmaceuticals. Where greater expression is desired over a larger area of tissue, increased dosages, or repeated dosages in a successive protocol of administrations, or several administrations to different locations may be practiced.

Pro-apoptotic agents and/or anti-proliferative agents, and pharmaceutical compositions containing the same, can be provided in kits, with suitable instructions and other necessary reagents, in order to conduct the treatment methods as described above. The kit can also contain, depending on the particular method and mode of administration used, suitable labels and other packaged reagents and materials (i.e. buffers and the like).

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

III. Experimental

Example 1

Fluoroscopy and Delivery of Methylene Blue Dye to Canine Prostates

Fluoroscopy was performed on 4 individual canines (beagles) in order to evaluate and optimize catheter design. Contrast media was introduced into the urethra under pressure to determine the integrity of catheter balloons to contain a contrast media, to determine the volume needed to fill the urethra, and

to determine the amount of pressure needed to fill the prostatic ducts with a delivered solution. The animals were premedicated with valium and ketamine and placed under general anesthesia using isoflurane. A
5 lubricated urinary drug delivery catheter, with its dead space filled with a contrast agent, was introduced through the urethra and into the bladder. The locating balloon at the bladder neck of the catheter was inflated with 6.0 mL of saline. Time of
10 catheter insertion was noted, and the catheter was slightly withdrawn to occlude the bladder. The distal urethral balloon of the catheter was then inflated with 0.75 mL of saline while maintaining tension on the catheter. The inflation of both the proximal and
15 distal balloons of the catheter isolates the prostatic portion of the canine urethra. An additional 2.5 mL of saline was then added to the bladder balloon, and a survey radiograph taken.

Sterile, nonionic radiographic contrast
20 agent meglumine iothalamate (50% isothalamate sodium in sterile water) was then introduced through the catheter and into the isolated prostatic urethra under low pressure. The contrast fluid was then slowly introduced in 0.5 mL increments until the prostatic
25 ducts were visible. The entire fluoroscopy procedure was recorded on videotape, and radiographs were taken at pressure increments deemed appropriate. Once the prostatic ducts were seen, an additional 0.5 mL of the contrast fluid was added. If a maximum pressure of 20
30 inches of water was reached in the prostatic urethra and the integrity of the catheter balloons was still maintained, the study was terminated whether or not the prostatic ducts were visible. A successful catheter design was indicated when the contrast media
35 was prevented or minimized from flowing down the distal urethra and/or into the bladder, and when the

contrast media was observed as being instilled within the prostatic ducts.

After fluoroscopy was completed, the catheter was removed and the animal was allowed to recover from anesthesia. It is expected that the recovering animals suffered no, or minimal pain, although the subjects were administered an analgesic if they appeared to have pain. The animals were also given 40,000 IU/kg of a mixture of procaine and benzathine penicillin via intramuscular injection to help minimize infection caused by bacteria that may be flushed into the prostatic ducts as a result of the above procedures.

Within one week after the fluoroscopy procedure, two of the animals were premedicated with valium and ketamine and placed under general anesthesia using isoflurane. Methylene blue was then delivered by transurethral catheter to the prostate using only pressure (passive delivery). A sterile drug delivery catheter dead space was filled with methylene blue dye prior to insertion into the urethra. The catheter was inserted into the urethra, and the balloons inflated as described above with respect to the fluoroscopy procedure. The methylene blue dye solution (5 mg/mL concentration) was introduced into the isolated prostatic urethra at an optimal pressure determined by the fluoroscopy experiment, using sufficient pressure to penetrate the prostatic ducts. The pressure was maintained for 20-30 minutes. After delivery, the animals were sacrificed with an overdose of phenobarbital (100 mg/kg, administered IV) and the entire prostate removed for drug quantification.

Also within one week of the fluoroscopy procedure, the remaining animals (n=2) were premedicated with valium and ketamine and placed under general anesthesia using isoflurane. By the following

procedure, methylene blue dye was delivered to the prostate of each animal by a combination of passive (pressure) and active (phoresis) drug delivery techniques. A sterile drug delivery catheter dead space was filled with methylene blue dye prior to insertion into the urethra. The catheter, containing a phoretic electrode, was inserted into the urethra and the balloons inflated as described in the above fluoroscopy procedure. A return electrode was placed on a shaved portion of the lateral abdomen. The methylene blue dye solution (5 mg/mL) was introduced into the isolated prostatic urethra at a pressure that was determined sufficient to penetrate the prostatic ducts by fluoroscopy. A low level of electrical current of about 5 to 10 mA was applied via the catheter to transport the charged drug into the prostatic tissue. The process was carried out for 20 to 30 minutes. After delivery, the animals were sacrificed with an overdose of phenobarbital (100 mg/kg IV) and the prostates removed for drug quantification.

The results of the fluoroscopy procedures were as follows. In the first animal (dog #1), application of pressure at 29 inches of water resulted in the point at which prostatic flair was seen (when the contrast dye was noted in the prostate ducts). For the second animal (dog #2), a pressure of 20 inches of water brought about prostatic flair, for the third animal (dog #3), a pressure of 18 inches of water produced prostatic flair, and, for the fourth animal (dog #4), a pressure of 25 inches of water was need to bring about prostatic flair.

Intense methylene blue staining of urethral and periurethral structure was observed as a result of both the passive (pressure-assisted), and active (pressure- and phoretic-assisted) dye delivery experiments. Of the 4 animals tested, dog #1 and dog

#4 received 22 and 23 minutes of active drug delivery, respectively, and dog #2 and dog #3 received 5 and 8 minutes of active drug delivery, respectively. The remainder of the 30 minute drug delivery for each dog was accomplished by passive drug delivery. There was no clear evidence that dogs #1 and #4 had better drug delivery than dogs #2 and #3, so that no conclusions can be drawn concerning which drug delivery protocol is most effective in canines.

Upon histological examination, significant staining of the glandular prostate was observed in dogs #3 and #4, and in these two animals, focal methylene blue staining of the glandular prostate was distributed, in a radial manner, from the urethra to the external prostatic capsule. Further, microscopic evidence showed an absence of coagulative changes in the subadjacent connective tissue which suggested that no thermal or electrical injury was present.

20

Example 2Delivery of Lidocaine to Canine Prostates
by Passive and Active Transurethral Catheterization

Two canines (beagles #47 and #52) were sedated with acepromazine and placed under general anesthetic using isoflurane. Prior to inserting catheters into the urethra, dead space in the catheter was filled with 4% lidocaine. The sterile, lubricated, drug delivery catheter possessing an electrode was introduced through the urethra and into the bladder. The bladder neck locating balloon was then inflated with 6 mL of saline. The time of catheter insertion was noted. The catheter was withdrawn slightly to occlude the bladder, and, while maintaining tension on the catheter, the distal urethral balloon was inflated with 0.75 mL of saline, thereby isolating the prostatic portion of the canine urethra. A return electrode was placed on a shaved

portion of the lateral abdomen. The lidocaine agent was then introduced incrementally into the isolated prostatic urethra to achieve a pressure of 25 inches of water. This pressure had previously been
5 determined sufficient to penetrate the prostatic ducts. A low level of electrical current of 5 mA was applied via the catheter to assist in the transport of the charged lidocaine agent into the prostatic tissue. This process was carried out simultaneously with the
10 application of pressure over a period of 30 minutes. The pressure was maintained at between 20 and 25 inches of water by incremental addition of the lidocaine solution.

After delivery of the lidocaine, the two
15 animals were sacrificed with an overdose of pentobarbital (100 mg/kg by IV injection) and the entire prostate of each animal was removed for inspection. The two prostatic lobes were separated by a longitudinal cut, and each lobe was sectioned into
20 six pieces for a total of 12 pieces per prostate to represent 3 periurethral, and 3 subcapsular zones of each prostate lobe. These prostate zones were analyzed by HPLC for total lidocaine concentration, and the urethral segment and representative segments
25 of the remainder of the prostates were prepared for histological analysis.

The results of the study showed significant lidocaine in the prostate of dog #52, indicating that the agent was successfully delivered via iontophoresis
30 and intraprostatic pressure throughout the entire glandular structure. The mucosal surface of the prostatic urethra exhibited mild to moderate mechanical effacement of urethral epithelial cells with sparing of basilar epithelial structures in most
35 regions. There was no distinct evidence of significant thermal or electrical damage to any submucosal connective tissue component. Dog #47 also

had detectable levels of lidocaine in the prostate, but in considerably less quantity as compared with dog #52. This result may be attributed to displacement of the catheter during delivery, resulting in balloon obstruction and occlusion of the prostatic ductal entry at the colliculus seminalus.

The above study was repeated using a marker gene construct to assess delivery of such agents to the prostate using the methods of the invention. The gene construct, which was administered via an iontophoretic-assisted transurethral catheter, contained the herpes simplex virus thymidine kinase gene (HSV-TK) under regulatory control of the cytomegalovirus (CMV) promoter, and also included the CMV early intron, a bovine growth hormone polyadenylation signal, and a PUC-derived kanamycin resistance gene. The results of the study were negative.

20

Example 3

In vitro Toxicity of BE4x4 in DU-145 Cells

In order to assess the toxicity of the polyamine analog BE4x4 on the human prostate cancer cell line DU-145 (ATCC Accession No. ATB 81, (1977) Cancer Res. 37:4049-4058), the following study was carried out.

DU-145 cells were maintained in minimal essential media supplemented with 10% fetal bovine serum (MEM/10% FBS). For the toxicity assay DU-145 cells were plated in a 96-well flat bottom plate at a density of 5000 cells/well in 100 μ L of the MEM/10% FBS. The apoptotic agent BE4x4 (N1, N14-bis(ethyl)-amino-5, 10-diazatetradecane) was dissolved in water at a concentration of 20 mM. The BE4x4 stock solution was diluted 1:5 in MEM/10% FBS, and from this a 1:2 serial dilution was made in MEM/10% FBS. 6 hours after plating of the cells, 5 μ L MEM/10% FBS,

containing various dilutions of the BE4x4 agent, was added to the cells in two sets of duplicates (final concentrations ranging from 200 μ M, 100 μ M, and down to 0.4 μ M). The cells were incubated at 37°C in 5% CO₂ for 7 days. One set of duplicates received a second dose of the same BE4x4 concentration on day 3 after plating.

Cytotoxicity was determined 7 days after exposure using the WST-1 reagent according to the manufacturers procedure (Boehringer Mannheim, Indianapolis, IN). In a separate experiment, DU-145 cells were plated in a 6-well plate, and, before addition of 20 μ M or 100 μ M of the BE4x4, the cells were pretreated with 100 μ M of the Caspase inhibitor peptide z-VAD-fmk (Enzyme System Products, Dublin, CA). After three days a second dose of z-VAD-fmk was added to the respective dishes.

The results of the cytotoxicity assay are depicted in Figure 1. As can be seen, the BE4x4 pro-apoptotic agent is clearly cytotoxic to the DU-145 cells, with an IC-50 of approximately 5 μ M. Cytotoxicity was first detected at day 5 after addition of the BE4x4 composition. Cytotoxicity was first determined by observing morphological changes in the cultured cells.

At Day 7 post administration of the BE4x4 composition, most cells at the higher doses (100-200 μ M) had died, and no normal, healthy-appearing cells could be identified from the cultures. A single administration of the composition was sufficient to induce cytotoxicity, suggesting that BE4x4 is relatively stable during this period. BE4x4-induced toxicity was not inhibited by incubation with the Caspase inhibitor z-VAD-fmk, a peptide able to inhibit apoptosis in a number of model systems.

The fact that the Caspase inhibitor z-VAD-fmk was unable to block the toxic effects of

BE4x4 suggests that Caspases are not involved in this process, however, this does not exclude the conclusion that the cells die through apoptosis.

The above-described techniques can be used to assess the toxicity of other polyamine analogs for toxicity in DU-145 cells, or in other prostatic cancer cell lines, such as PC-3 (ATCC Accession No. CRL 1435) or LnCAP (ATCC Accession No. CRL 1740).

10

Example 4

Local Delivery of Pro-Apoptotic or Anti-Proliferative Agents to the Rat Prostate

In order to assess the effect of pro-apoptotic agents on prostate weight, the following studies were carried out. Male rats, 10 to 16 weeks of age, were surgically opened to expose the two ventral lobes of the prostate. Each prostate was then injected with 200 μ L of a pro-apoptotic composition using a 20 gauge needle. The animals were then sutured closed. After various time periods, the experimental animals were sacrificed, and the urinary bladder and ventral lobes of the prostate removed and weighed. The tissues were then fixed in 10% formalin buffer, and histopathological slides prepared for further evaluation.

In particular, the following pro-apoptotic agents were assessed: compound 11013 (4-pentoxo-1,2-naphthoquinone); compound 11019 (4-cyclohexymethoxy-1,2-naphthoquinone); and compound 11053 (bis-6[(1,2-naphthoquinone-4-yl)oxy]hexyloxy carbonate). The following anti-proliferative agent was also assessed: compound BE4x4 (N1, N14-bis(ethyl)-amino-5, 10-diazatetradecane).

Experimental groups were established as follows:

Group (1): 2.0 mM of compounds 11019 and 11013, and prostates harvested on Day 2 and Day 5 post administration;

5 Group (2): 0.2 mM of compounds 11019 and 11013, and prostates harvested on Day 2 and Day 5 post administration;

Group (3): 0.5 and 2.0 mM of compounds 11019 and 11013, and prostates harvested on Day 2 and Day 5 post administration;

10 Group (4): 0.5 and 2.0 mM of compound BE4x4, and prostates harvested on Day 8 post administration;

Group (5): 2.0 mM of compound 11019, and prostates harvested on Day 5 post administration;

15 Group (6): 0.5, 2.0, and 10.0 mM of compound BE4x4, and prostates harvested on Day 16 post administration;

Group (7): 0.5, 2.0, and 10.0 mM of compound BE4x4, and prostates harvested on Day 8 post administration; and

20 Group (8): 2.0 mM of compound 11019, and prostates harvested on Day 5 post treatment.

A significant decrease in prostate weight (30 to 40% decrease) was observed in the Group 4, 5, 6 and 8 animals that were treated with either compound
25 BE4x4 or 11019 at the 2.0 mM range. In these Groups, control animals had mean prostate weights of approximately 0.8 g or larger. In Groups 3 and 7, where the mean prostate weight of the control groups were less than 0.8 g, no significant difference
30 between control and experimental animals was observed. When the pro-apoptotic or anti-proliferative agents were administered in doses of less than 2.0 mM, no significant decrease in prostate weights was observed. In the Group 6 animals, where 10 mM doses of the anti-proliferative agent BE4x4 were administered, the
35 animals did not gain or lose weight during the test periods. Further, the 10 mM dose of BE4x4 brought

about the same degree of prostate reduction as seen with the 2.0 mM dose of the same agent.

Thus, novel methods for treating prostatic disorders by delivery of pro-apoptotic and/or anti-proliferative agents to the prostate, and kits suitable for practicing the methods, have been described. Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit and the scope of the invention as defined by the appended claims.

Deposits of Strains Useful in Practicing the Invention

A deposit of biologically pure cultures of the following materials was made with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland. The accession number indicated was assigned after successful viability testing, and the requisite fees were paid. The deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of viable cultures for a period of thirty (30) years from the date of deposit. The materials will be made available by the ATCC under the terms of the Budapest Treaty, and subject to an agreement between Chiron Corporation and the ATCC, which assures permanent and unrestricted availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 U.S.C. §122 and the Commissioner's rules pursuant thereto (including C.F.R. §1.12 with particular reference to 886 OG 638).

These deposits are provided merely as convenience to those of skill in the art, and are not an admission that a deposit is required under 35

U.S.C. §112. The nucleic acid sequences of these
plasmids, as well as the amino acid sequences of the
polypeptides encoded thereby, are incorporated herein
by reference and are controlling in the event of any
5 conflict with the description herein. A license may
be required to make, use, or sell the deposited
materials, and no such license is hereby granted.
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§1.808(b), all restrictions imposed on the
10 availability to the public of the deposited material
will be irrevocably removed upon the granting of the
patent.

Deposit Information:

15

<u>Name</u>	<u>Deposit Date</u>	<u>Accession No.</u>
<i>Escherichia coli</i> DH5a, pPNT	30 January 1996	69980
pCMV-Km-TK	28 February 1996	97452
20 pCMV-KmBgalepi	21 June 1996	97621

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Claims:

1. A method for treating benign prostatic hyperplasia (BPH) in a mammalian subject, comprising
5 delivering a pharmaceutical composition containing a therapeutically effective amount of a pro-apoptotic agent to the prostate of the subject.
2. The method of claim 1, wherein the pro-
10 apoptotic agent is a polypeptide selected from the group consisting of fas, fas ligand, fadd, fap-1, tradd, faf, rip, reaper, apoptin, interleukin-2 converting enzyme (ICE), FADD-homologous ICE-CED3-like protease (FLICE), bax, P53, TNF receptor type-I, TNF-
15 alpha, and cytochrome-c.
3. The method of claim 1, wherein the pro-apoptotic agent is a compound selected from the group consisting of phenyl buterate, phenyl acetate, 1-
20 phenyl-2-decanoyl amino-3-morpholino-1-propanol (PDMP), thapsigargin, 4-substituted-1,2-naphthoquinones, and pharmaceutically acceptable salts of said compounds.
- 25 4. The method of claim 3, wherein the pro-apoptotic agent is a 4-substituted-1,2-naphthoquinone.
5. The method of claim 4, wherein the pro-apoptotic agent is a 4-alkoxy-1,2-naphthoquinone or a
30 pharmaceutically acceptable salt thereof.
6. The method of claim 5, wherein the 4-alkoxy-1,2-naphthoquinone is selected from the group consisting of 4-pentoxy-1,2-naphthoquinone, 4-
35 cyclohexymethoxy-1,2-naphthoquinone and bis-6[(1,2-naphthoquinone-4-yl)oxy]hexyloxy carbonate.

7. A method for treating benign prostatic hyperplasia (BPH) in a mammalian subject, comprising delivering a pharmaceutical composition containing a therapeutically effective amount of an anti-proliferative agent to the prostate of the subject.

8. The method of claim 7, wherein the anti-proliferative agent is a polyamine analog.

9. The method of claim 8, wherein the polyamine analog has the general formula:

(I) $R_1-NH-(CH_2)_w-NH-(CH_2)_x-NH-(CH_2)_y-NH-(CH_2)_z-NH-R_2$,
wherein R_1 and R_2 are hydrocarbon chains having 1 to 5 carbons, and w , x , y and z are integers of 1 to 10.

10. The method of claim 9, wherein the polyamine analog is N1, N14-bis(ethyl)-amino-5, 10-diazatetradecane or a pharmaceutically acceptable salt thereof.

11. The method of claim 1 or 7, wherein the pharmaceutical composition is delivered transurethrally to the prostate or periprostate space of the subject.

12. The method of claim 1 or 7, wherein the pharmaceutical composition is delivered by direct injection into the prostate of the subject.

13. The method of claim 1 or 7, wherein the pharmaceutical composition is delivered by catheter into the urethra of the subject.

14. The method of claim 13, wherein catheter delivery of the pharmaceutical composition is assisted by phoresis.

15. The method of claim 13, wherein catheter delivery of the pharmaceutical composition is assisted by pressure.

5 16. A method for treating benign prostatic hyperplasia (BPH) in a mammalian subject, comprising delivering to the prostate of the subject a therapeutically effective amount of a polynucleotide molecule comprising a coding sequence for a pro-
10 apoptotic agent operably linked to control elements, whereby said coding sequence can be transcribed and translated in the subject *in vivo*.

 17. The method of claim 16, wherein the
15 pro-apoptotic agent is selected from the group consisting of fas, fas ligand, fadd, fap-1, tradd, faf, rip, reaper, apoptin, interleukin-2 converting enzyme (ICE), FADD-homologous ICE-CED3-like protease (FLICE), bax, P53, TNF receptor type-I, TNF-alpha, and
20 cytochrome-c.

 18. The method of claim 17, wherein the pro-apoptotic agent is fas or fas ligand.

25 19. The method of claim 16, wherein the polynucleotide molecule is present in a vector.

 20. The method of claim 16, wherein the polynucleotide molecule is delivered transurethrally
30 to the prostate or periprostate space of the subject.

 21. The method of claim 16, wherein the polynucleotide molecule is delivered by direct injection into the prostate of the subject.

35

22. The method of claim 16, wherein the polynucleotide molecule is delivered by catheter into the urethra of the subject.

5 23. A kit for use in treating benign prostatic hyperplasia (BPH) in a mammalian subject, comprising:

 (a) a pharmaceutical composition containing a therapeutically effective amount of a pro-apoptotic agent; and

10 (b) instructions for treating the subject using the pharmaceutical composition.

 24. A kit for use in treating benign prostatic hyperplasia (BPH) in a mammalian subject, comprising:

 (a) a pharmaceutical composition containing a therapeutically effective amount of an anti-proliferative agent; and

20 (b) instructions for treating the subject using the pharmaceutical composition.

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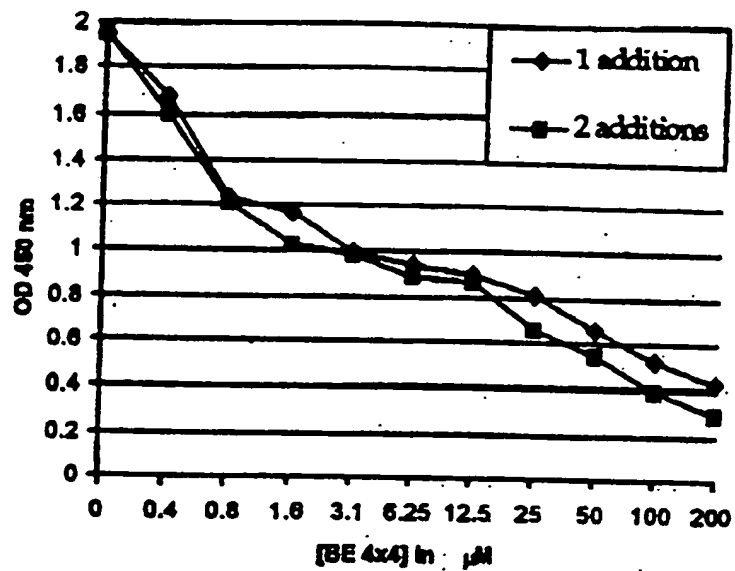


FIGURE 1



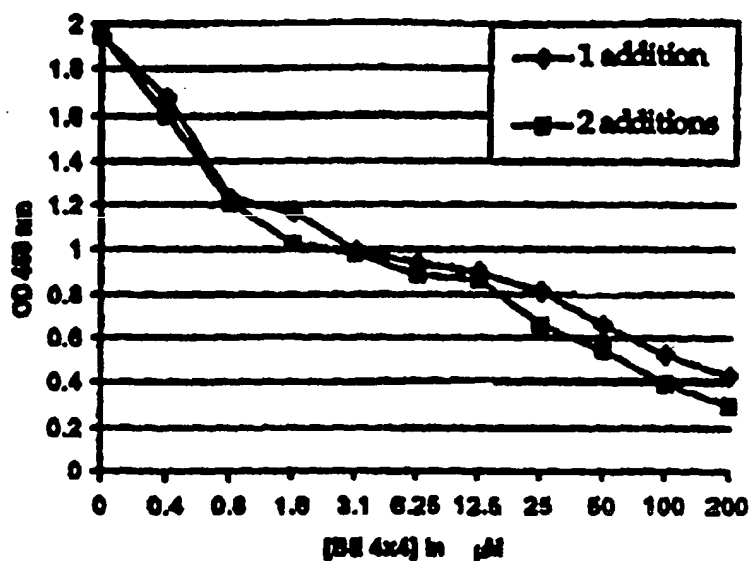
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 38/17, 38/19, 38/48, 38/44, 31/22, 31/535, 31/12, 48/00, 31/785		A3	(11) International Publication Number: WO 97/31611
			(43) International Publication Date: 4 September 1997 (04.09.97)
(21) International Application Number: PCT/US97/03228		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG). Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM). European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 28 February 1997 (28.02.97)			
(30) Priority Data: 60/012,672 1 March 1996 (01.03.96) US 60/012,817 5 March 1996 (05.03.96) US 60/021,539 11 July 1996 (11.07.96) US 08/807,181 27 February 1997 (27.02.97) US			
(71) Applicant: CHIRON CORPORATION [US/US]; 4560 Horton Street, Emeryville, CA 94608 (US).		Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(72) Inventor: HUNG, David, T.; 460 Yerba Buena Avenue, San Francisco, CA 94124 (US).			
(74) Agents: SAVEREIDE, Paul, B.; Chiron Corporation, Intellectual Property - R440, P.O. Box 8097, Emeryville, CA 94662-8097 (US) et al.		(88) Date of publication of the international search report: 13 November 1997 (13.11.97)	

(54) Title: TREATMENT OF BEGNIN PROSTATIC HYPERPLASIA

(57) Abstract

Novel methods for delivery of pro-apoptotic and/or anti-proliferative agents to the prostate gland for treatment of prostate disorders are disclosed. Kits for practicing the methods of the invention are also provided. The methods and kits of the invention can employ a catheter-based delivery system that uses phoresis, pressure, or both, to assist in the delivery of the pro-apoptotic and/or anti-proliferative agents to the prostate gland and/or surrounding regions.



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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/03228

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K38/17 A61K38/19 A61K38/48 A61K38/44 A61K31/22
A61K31/535 A61K31/12 A61K48/00 A61K31/785

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RITTMASER R.S. ET AL: "Evidence for atrophy and apoptosis in the prostates of men given Finasteride" JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, vol. 81, no. 2, February 1996, pages 814-819, XP002034672 see the whole document	1,11-15, 23
Y	---	2,16-22
Y	COLOMBEL M.: "Growth factors, cell proliferation and apoptosis in prostate adenoma" BIOMEDICINE & PHARMACOTHERAPY, vol. 48, no. 1 suppl, 1994, pages 25s-26s, XP002034673 see the whole document ---	2,16-22
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

8 July 1997

Date of mailing of the international search report

01.10.97

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INTERNATIONAL SEARCH REPORT

Intern. App. No.
PCT/US 97/03228

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SILVER, R.I. ET AL: "Androgen suppression therapy for benign prostatic hyperplasia" CURRENT OPINION IN UROLOGY, vol. 4, no. 1, 1994, pages 22-28, XP002034674 see the whole document ---	1,11-15, 23
X	WO 94 06910 A (CANJI INC ;SHEPARD H MICHAEL (US); KAN NANCY (US)) 31 March 1994 see page 7, line 33 - page 8, line 9 see page 13, line 25 - page 14, line 14 see claims 16-23 ---	1,2, 11-23
A	COHEN J.J.: "Apoptosis" IMMUNOLOGY TODAY, vol. 14, no. 3, 1993, pages 126-130, XP002034675 see the whole document ---	1,2
A	COHEN J.J.: "Exponential growth in apoptosis" IMMUNOLOGY TODAY, vol. 16, no. 7, 1995, pages 346-348, XP002034676 see the whole document ---	1,2
A	CHEMICAL ABSTRACTS, vol. 123, no. 25, 1995 Columbus, Ohio, US; abstract no. 332818, MIURA, M. ET AL: "Molecular mechanisms and signalling pathway of apoptosis" page 428; column 2; XP002034678 see abstract & Jikken Igaku, 1995, 13(16), 1824-7 (Japan) ---	1,2
A	US 5 007 897 A (KALB IRVIN M ET AL) 16 April 1991 see the whole document ---	11-15
A	EASTHAM, J.A. ET AL: "In vivo gene therapy with p53 or p21 adenovirus for prostate cancer" CANCER RESEARCH, vol. 55, 1995, pages 5151-5155, XP002034677 see the whole document ---	16-22
A	WO 95 23141 A (PFIZER ;ARNOLD LEE D (US)) 31 August 1995 see page 27, line 8 - line 17; claim 24 ---	1
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/03228

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 639 573 A (HOECHST AG) 22 February 1995 see page 6, line 39 - line 46; claim 17 ---	1
A	EP 0 688 766 A (HOECHST AG) 27 December 1995 see page 7, line 8 - line 15; claim 15 -----	1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 97/03228

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1-2,11-22
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Subject 1): claim 1,11-15,23 (all in part); and 2,16-22 (complete)
Subject 2): claim 1,11-15,23 (all in part); and 3-6 (complete)
Subject 3): claim 1,11-15 (all in part); and 7-10,24 (completely)

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1,11-15,23 (in part)
2,16-22 (complete)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/03228

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9406910 A	31-03-94	AU 676204 B AU 4929593 A CA 2144848 A EP 0672137 A JP 8504095 T	06-03-97 12-04-94 31-03-94 20-09-95 07-05-96
US 5007897 A	16-04-91	NONE	
WO 9523141 A	31-08-95	AU 2972795 A CA 2183655 A CN 1141633 A EP 0746554 A FI 963283 A HU 76291 A JP 9501953 T NO 963506 A PL 315941 A	11-09-95 31-08-95 29-01-97 11-12-96 22-08-96 28-07-97 25-02-97 22-10-96 09-12-96
EP 0639573 A	22-02-95	DE 4326005 A DE 4414316 A AU 6884494 A CA 2129301 A CN 1118347 A FI 943579 A HU 70547 A JP 7145149 A NO 942864 A NZ 264130 A ZA 9405734 A	09-02-95 26-10-95 16-02-95 04-02-95 13-03-96 04-02-95 30-10-95 06-06-95 06-02-95 21-12-95 07-03-95
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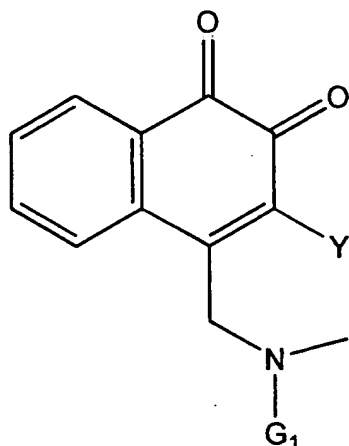
INTERNATIONAL SEARCH REPORT

Information on patent family members

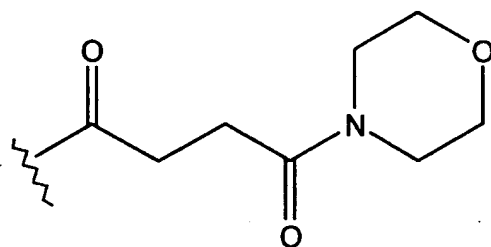
Intern. Patent Application No

PCT/US 97/03228

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0588766 A		PL 309168 A	27-12-95
		SK 80395 A	05-06-96
		ZA 9505027 A	08-02-96

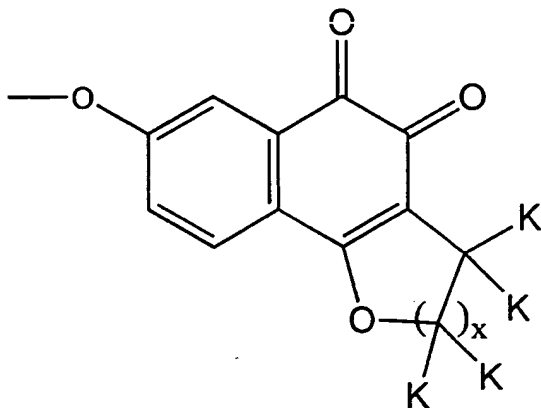


wherein Y is selected from the group consisting of -H, -F, -Br, -Cl, and -I; and wherein G₁ is selected from the group consisting of H, C₁-C₈ alkyl,

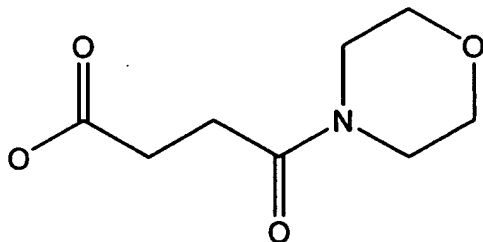


- 5 and -C(=O)-CH_nX_{3-n}, where n is an integer from 0 to 3 and X is selected from the group consisting of F, Cl, Br, and I; and the peptide is conjugated to the quinone via the amino group bearing G₁.

- 10 20. A composition according to claim 16, where the naphthoquinone is selected from the group of compounds of the formula



wherein x is 1 or 2; and each K is independently selected from the group consisting of H, C₁-C₈ alkyl, C₁-C₈ alkenyl, C₁-C₈ alkoxy, and



5

and where zero or two, but no more than two, vicinal K's in the molecule represent single electrons which form a pi bond, thus forming a double bond together with the existing sigma bond between the two adjacent carbons bearing the two vicinal K's.

10

21. A composition according to claim 15 wherein the naphthoquinone contains a primary or secondary amino group and is linked to the peptide by said amino group.

22. A composition according to claim 1, wherein the peptide substrate is a substrate of prostate specific antigen (PSA).

15

23. A composition according to claim 1, wherein the peptide sequence comprises SKLQ.

24. A composition according to claim 1, wherein the peptide sequence comprises SKLQL or SKLQ-β-alanine.

20

25. A composition according to claim 1, wherein the peptide sequence comprises HSSKLQ.

26. A composition according to claim 1, wherein the peptide substrate is a substrate of cathepsin B.

27. A composition according to claim 1, wherein the peptide sequence is X-P2-P1, where X is hydrogen, an amino-protecting group, or an amino-capping group attached to the N-terminus of P2; P2 is a hydrophobic amino acid; and P1 is a basic or polar amino acid.

28. A composition according to claim 1, wherein the peptide sequence is X-P2-P1-Y, where X is hydrogen, an amino-protecting group, or an amino-capping group attached to the N-terminus of P2; P2 is a hydrophobic amino acid; P1 is a basic or polar amino acid; and where Y is leucine, β -alanine, or a nonentity.

29. A composition according to claim 27, wherein X is a 4-morpholinocarbonyl group.

30. A composition according to claim 28, wherein X is a 4-morpholinocarbonyl group.

31. A composition according to claim 29, wherein P2 is selected from the group consisting of leucine, isoleucine, valine, methionine, and phenylalanine; and P1 is selected from the group consisting of lysine, arginine, glutamine, asparagine, histidine and citrulline.

32. A composition according to claim 30, wherein P2 is selected from the group consisting of leucine, isoleucine, valine, methionine, and phenylalanine; and P1 is selected from the group consisting of lysine, arginine, glutamine, asparagine, histidine and citrulline.

33. A composition comprising the polyamine analog conjugate of claim 1, and a pharmaceutically acceptable excipient.

34. A method of treating a disease characterized by the proliferation of prostate cells in an individual comprising administering to the individual a therapeutic amount of a polyamine analog conjugate of claim 1.

35. The method of claim 34, wherein the polyamine analog is conformationally restricted.

5 36. The method of claim 34, wherein the disease is prostatitis, benign prostate hyperplasia or prostate cancer.

37. The method of claim 34, wherein the individual is a human.

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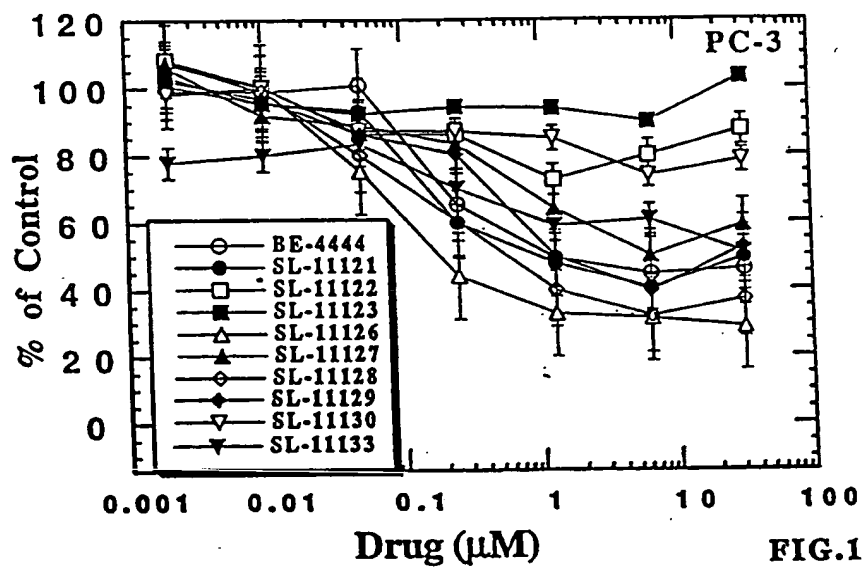
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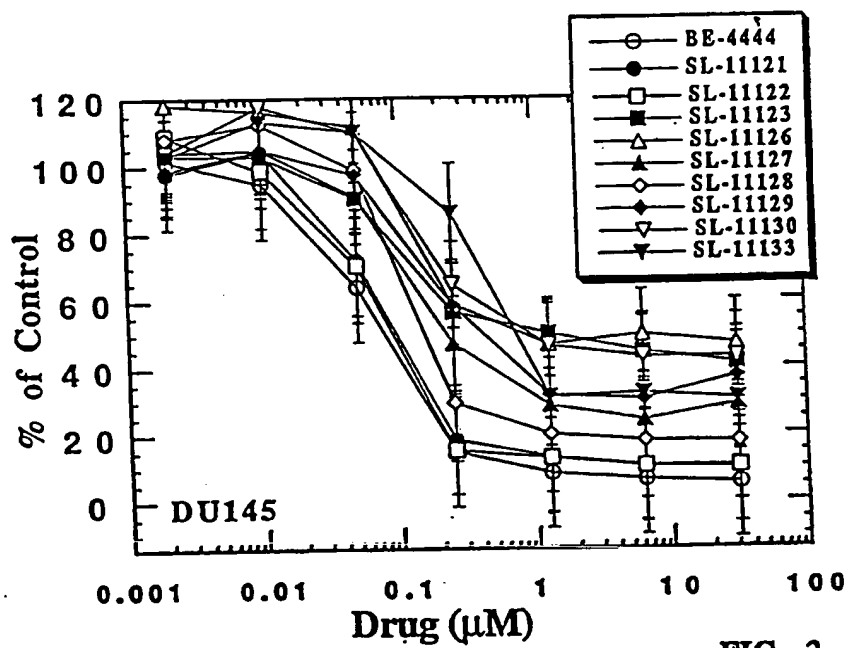


FIG. 2

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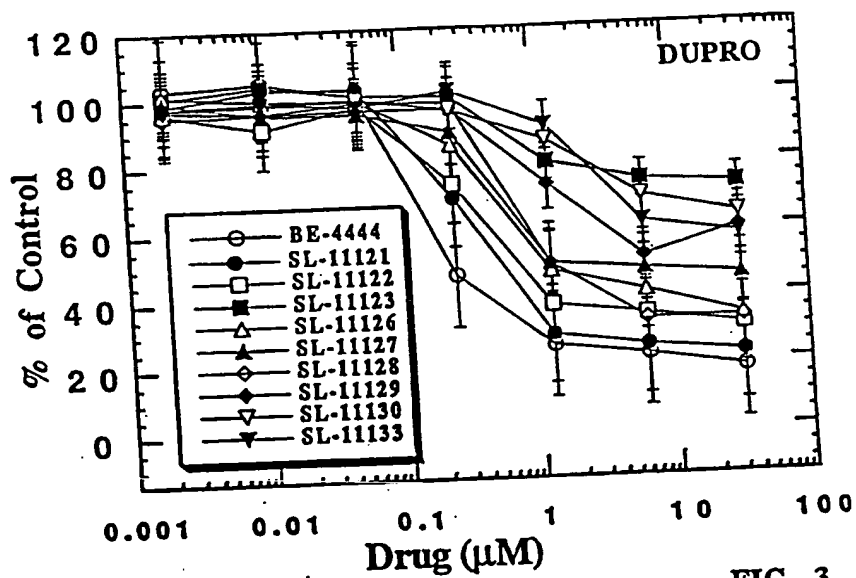


FIG. 3

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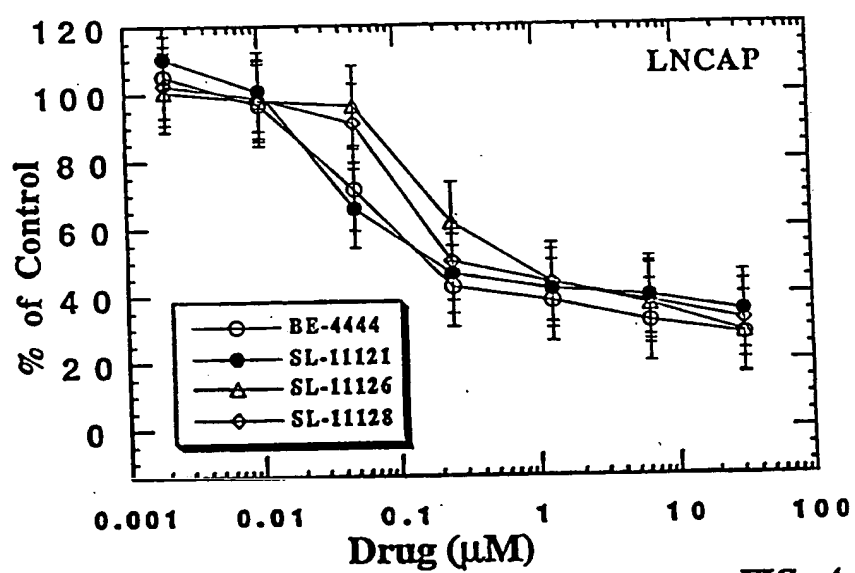
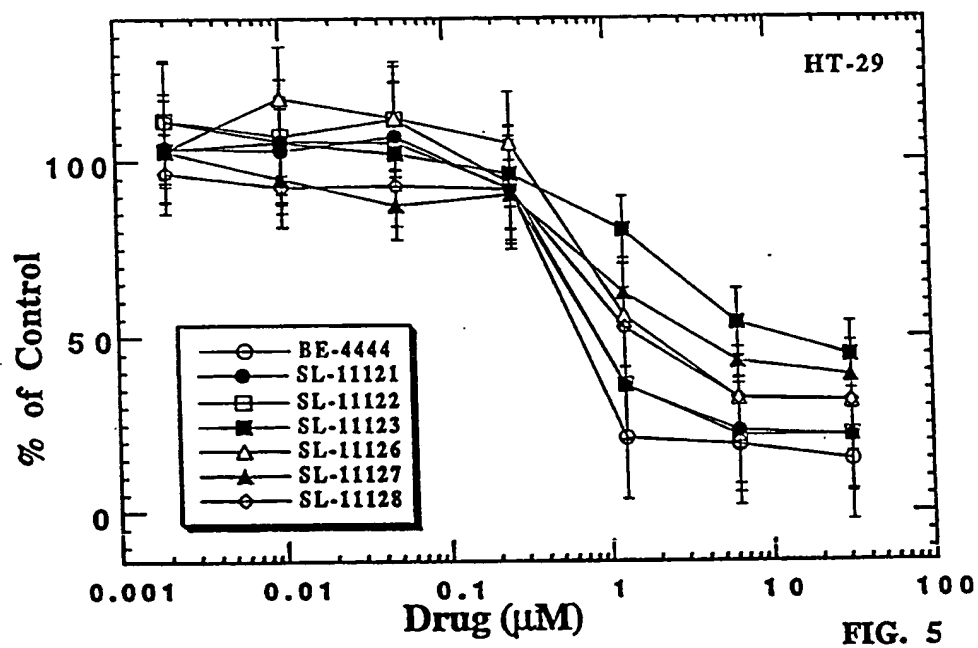


FIG. 4

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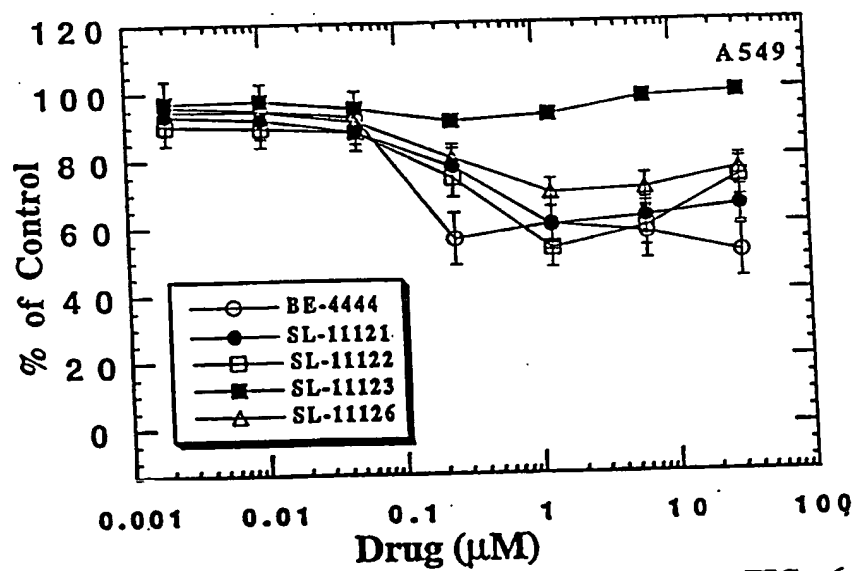


FIG. 6

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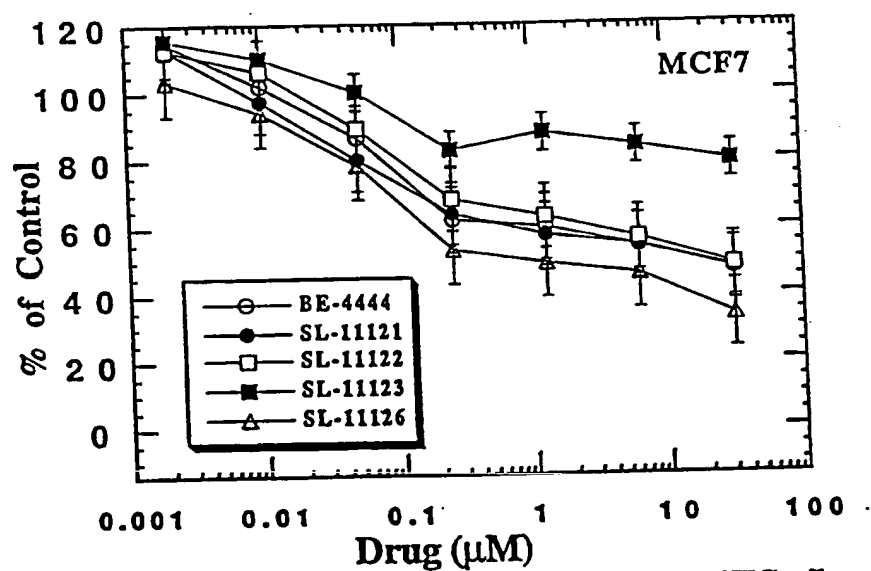


FIG. 7

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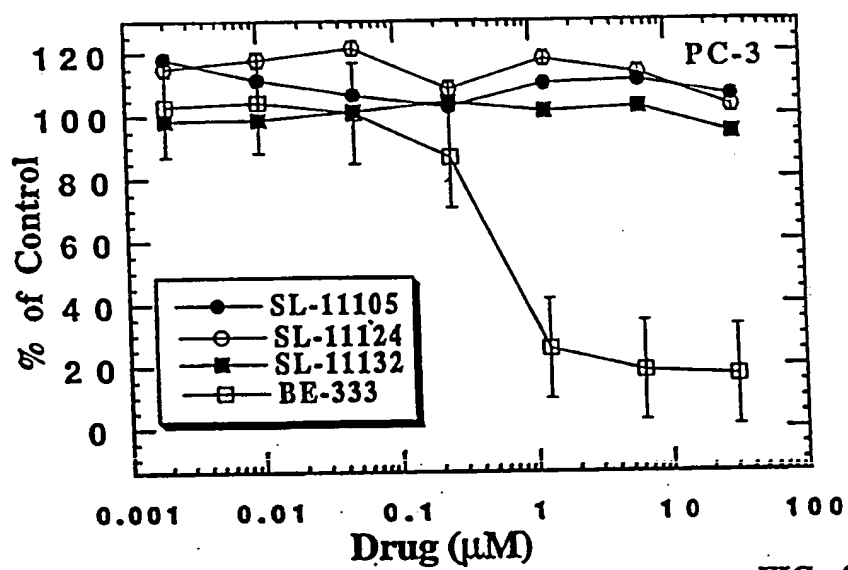


FIG. 8

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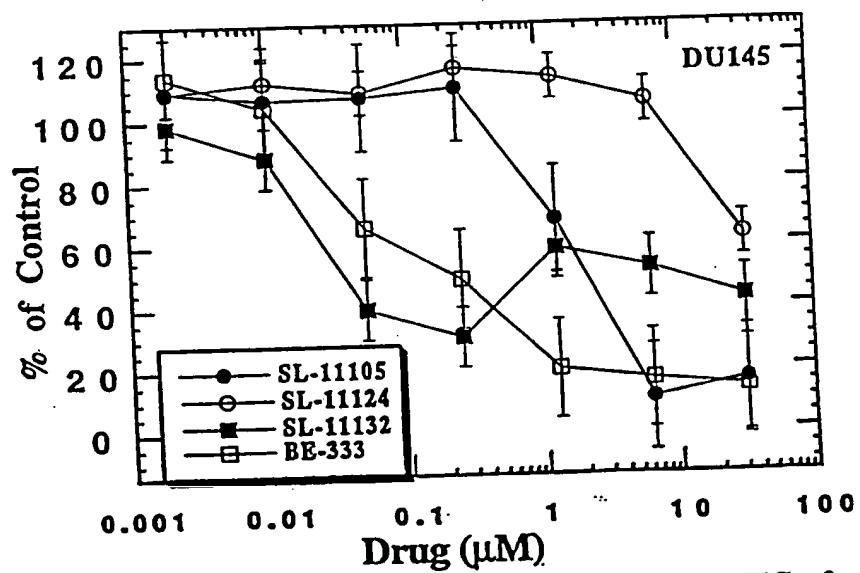


FIG. 9

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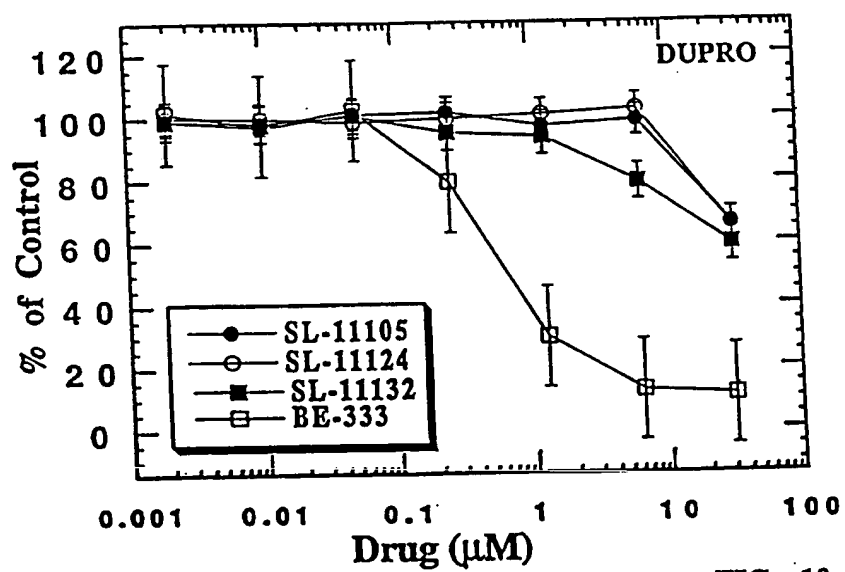


FIG. 10

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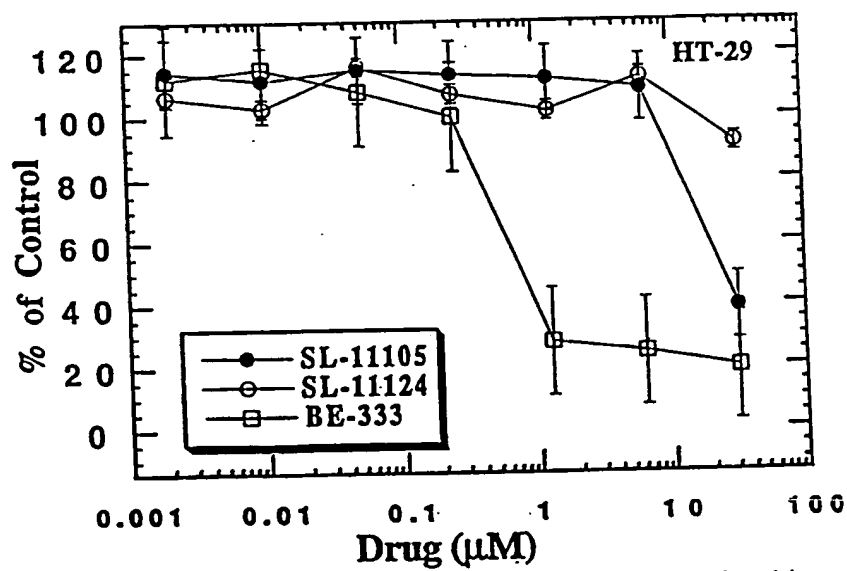


FIG. 11

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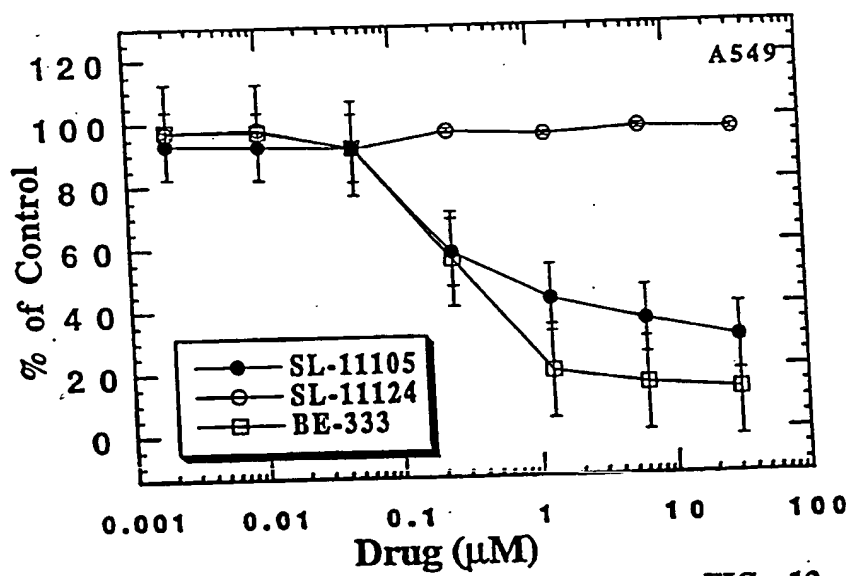


FIG. 12

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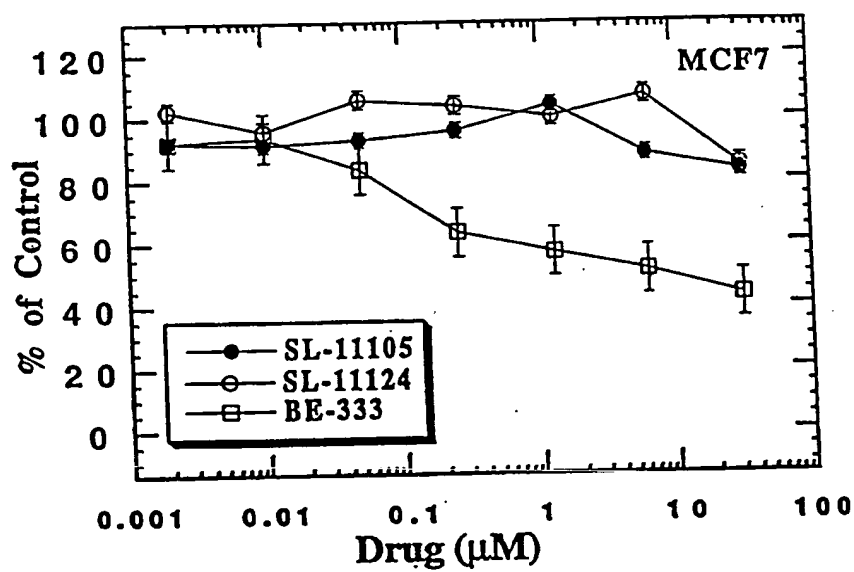


FIG. 13

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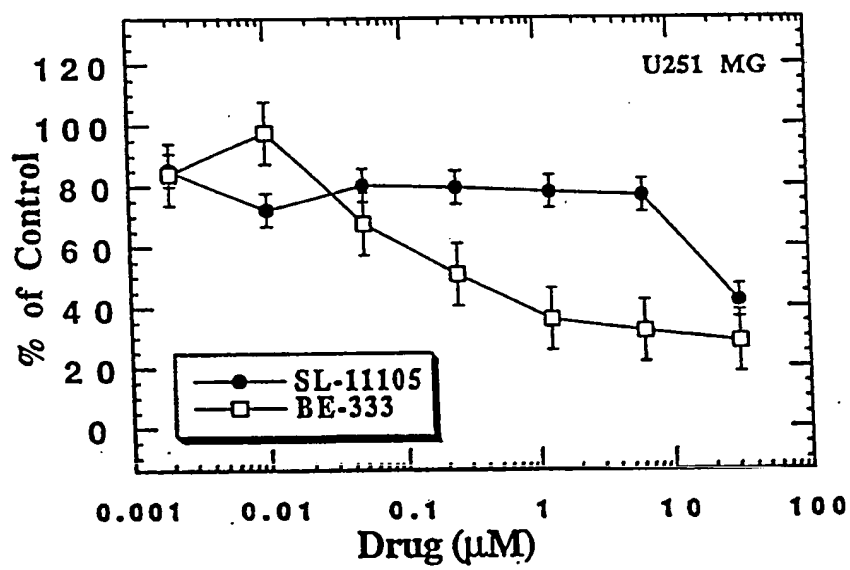


FIG. 14

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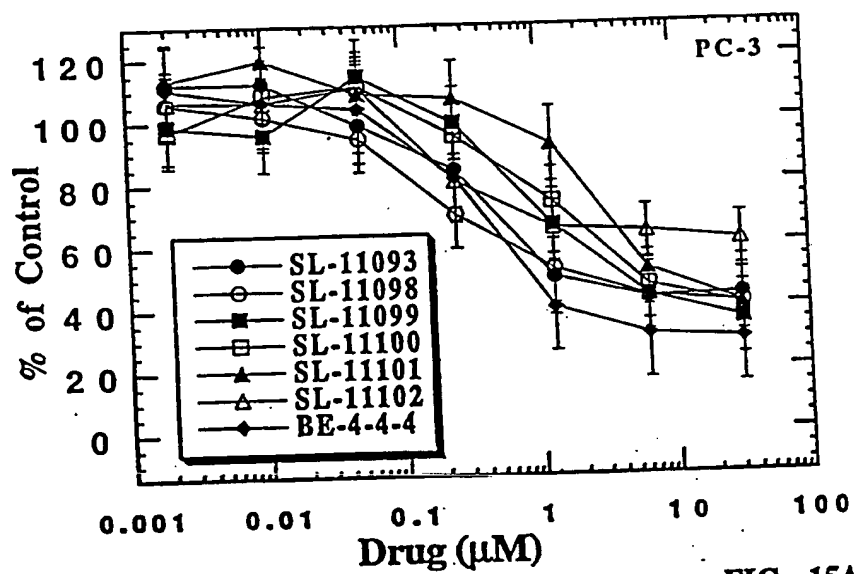


FIG. 15A

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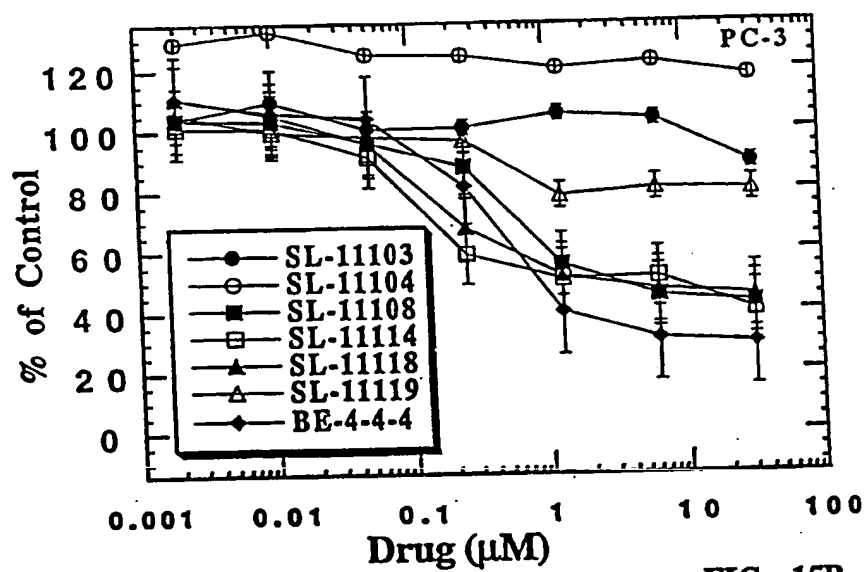


FIG. 15B

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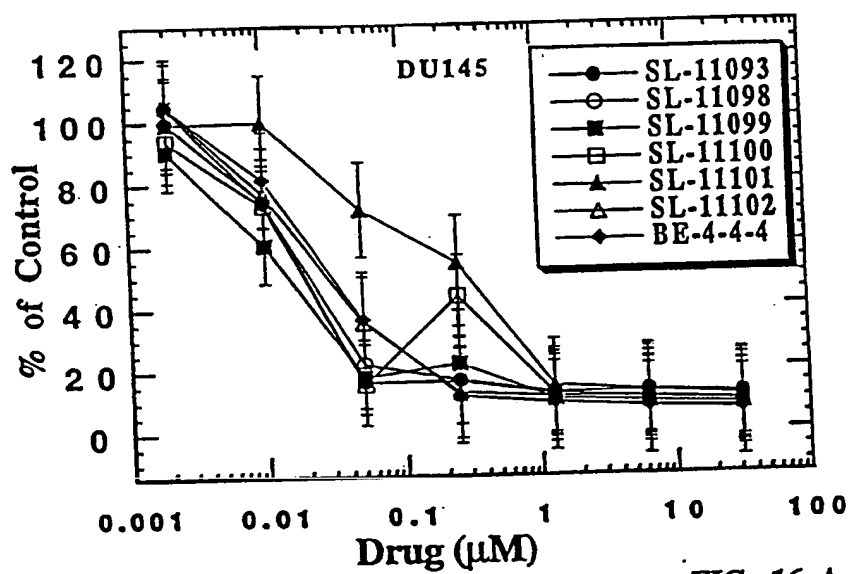


FIG. 16 A

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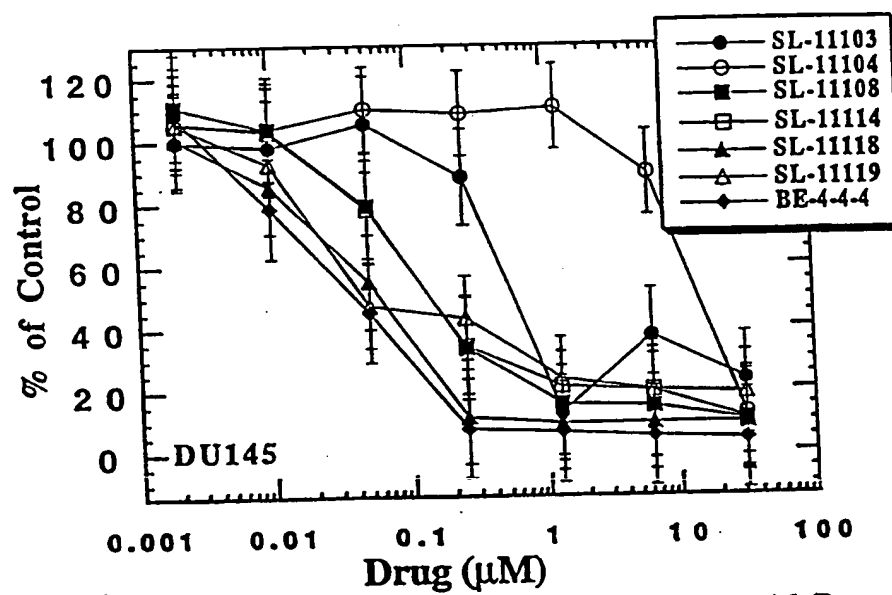
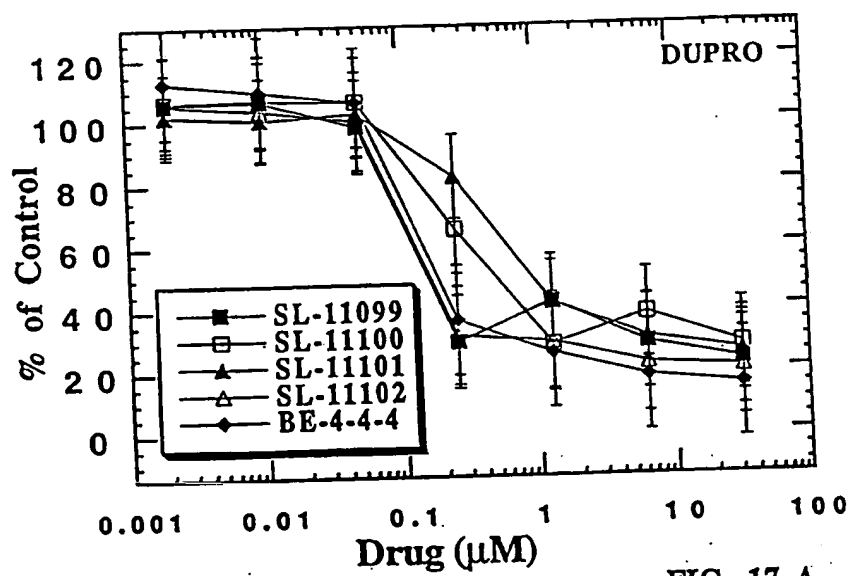


FIG. 16 B

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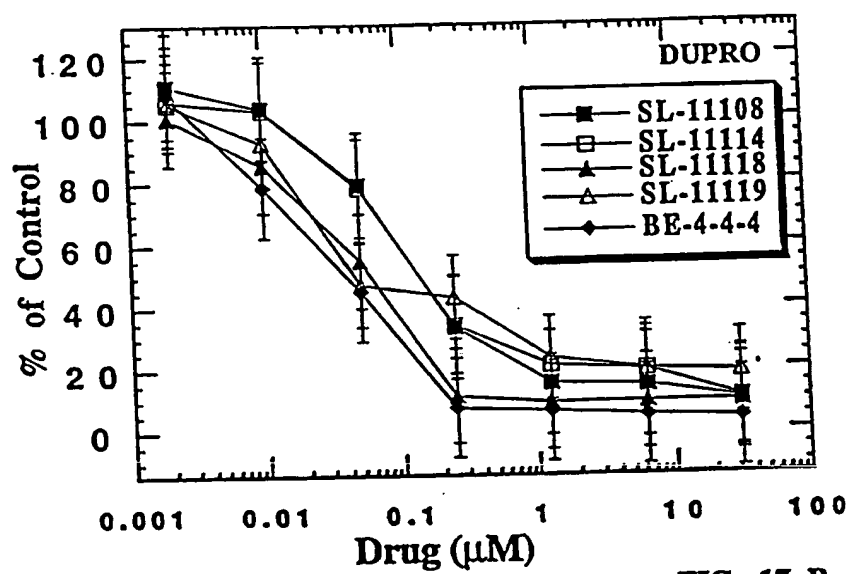


FIG. 17 B

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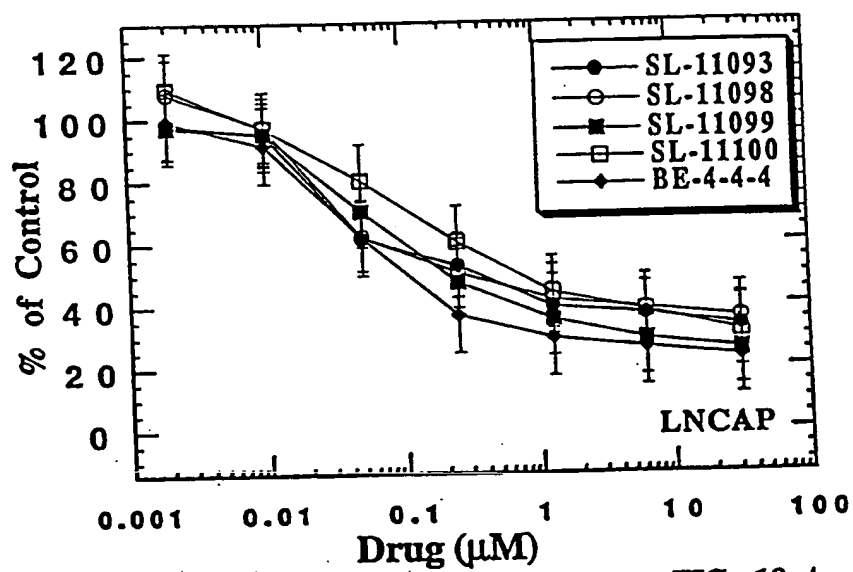


FIG. 18 A

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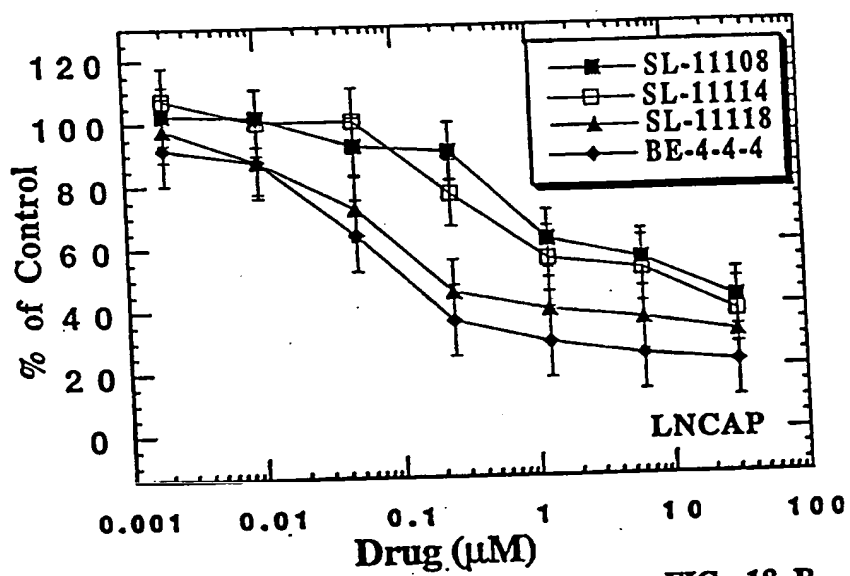


FIG. 18 B

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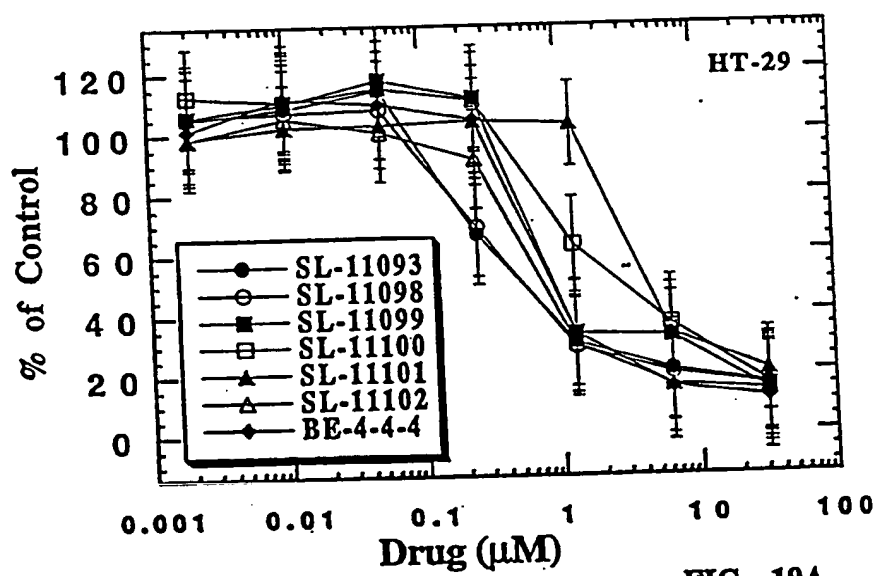


FIG. 19A

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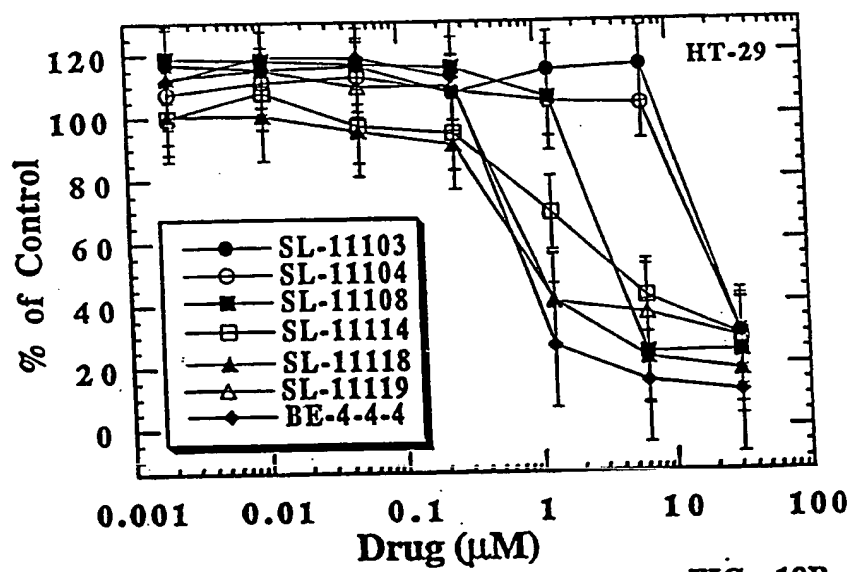


FIG. 19B

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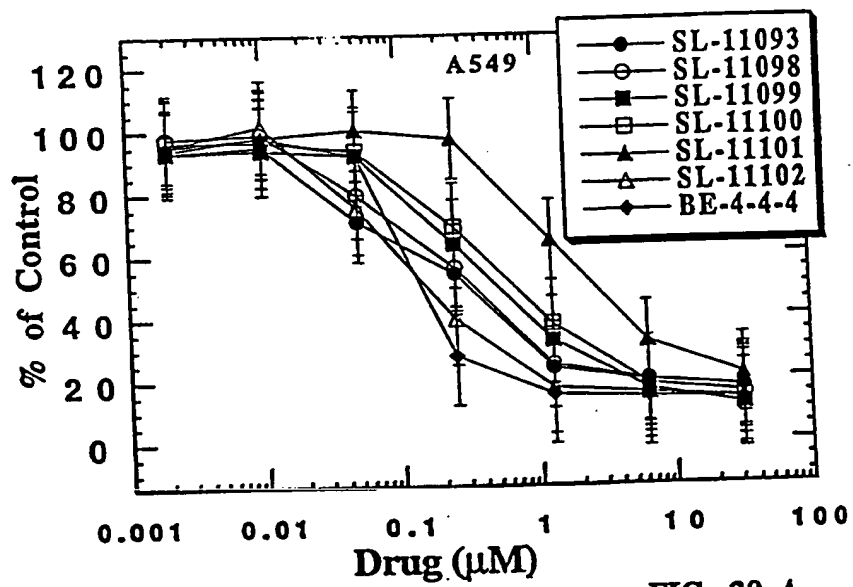


FIG. 20 A

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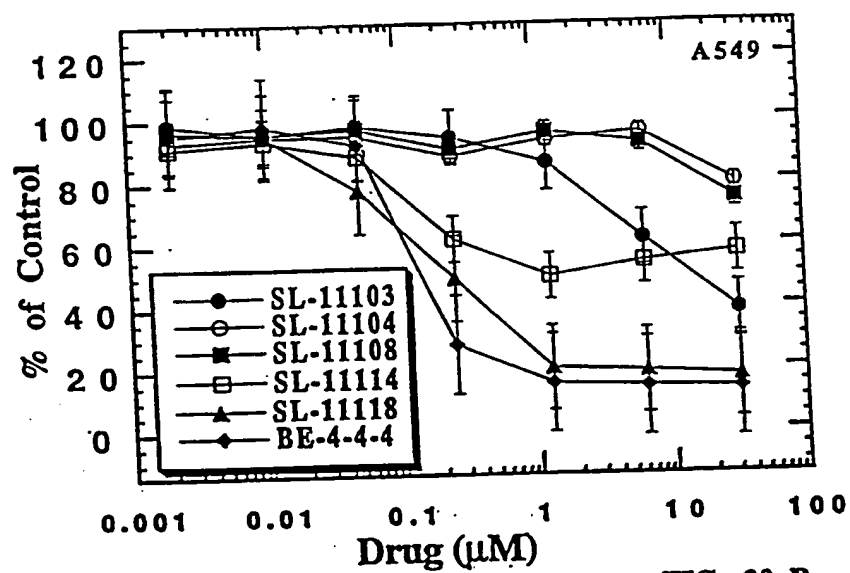


FIG. 20 B

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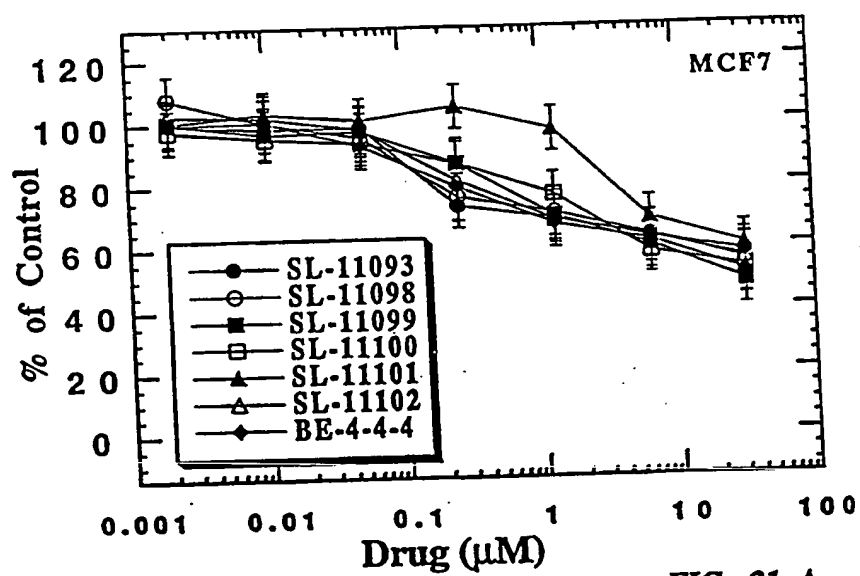


FIG. 21 A

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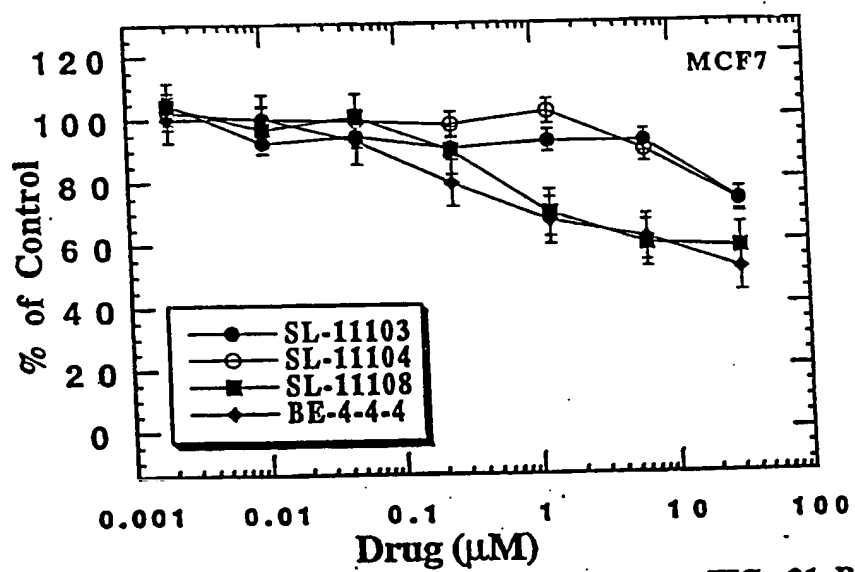


FIG. 21 B

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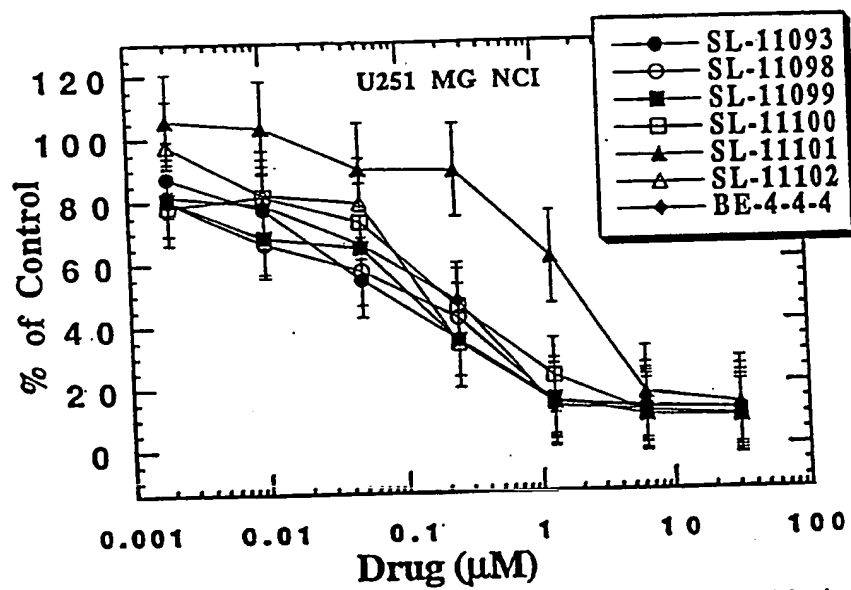


FIG. 22 A

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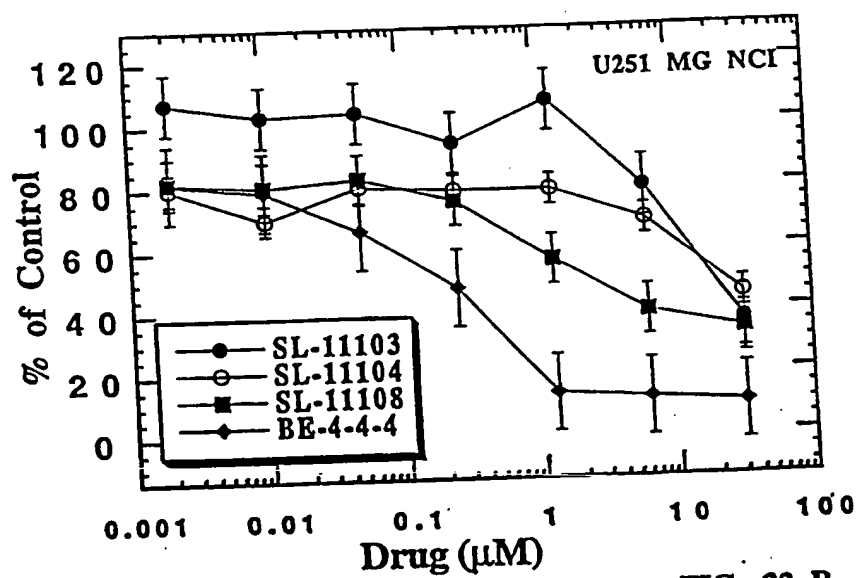


FIG. 22 B

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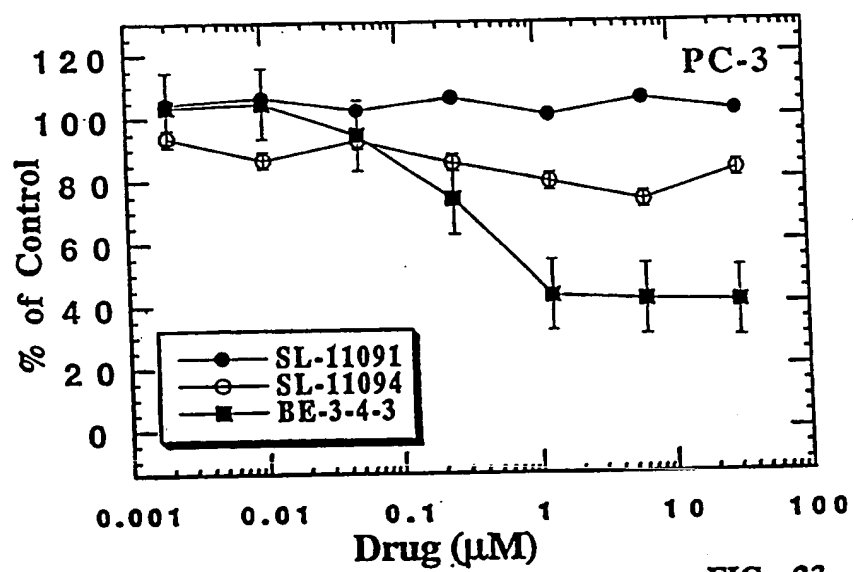


FIG. 23

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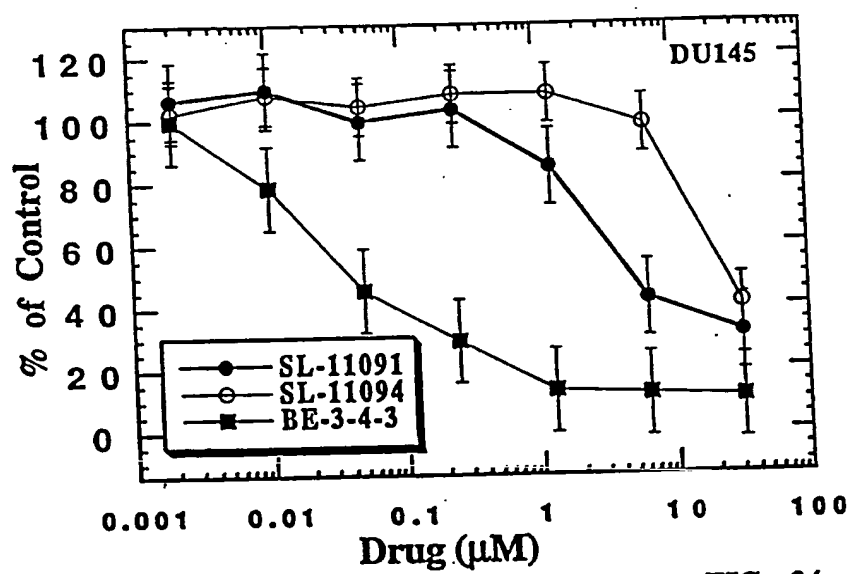


FIG. 24

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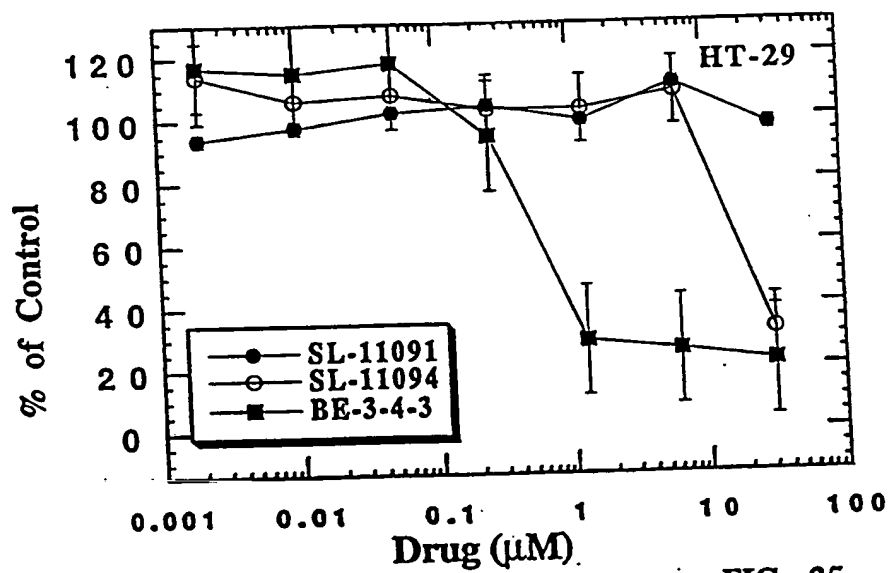


FIG. 25

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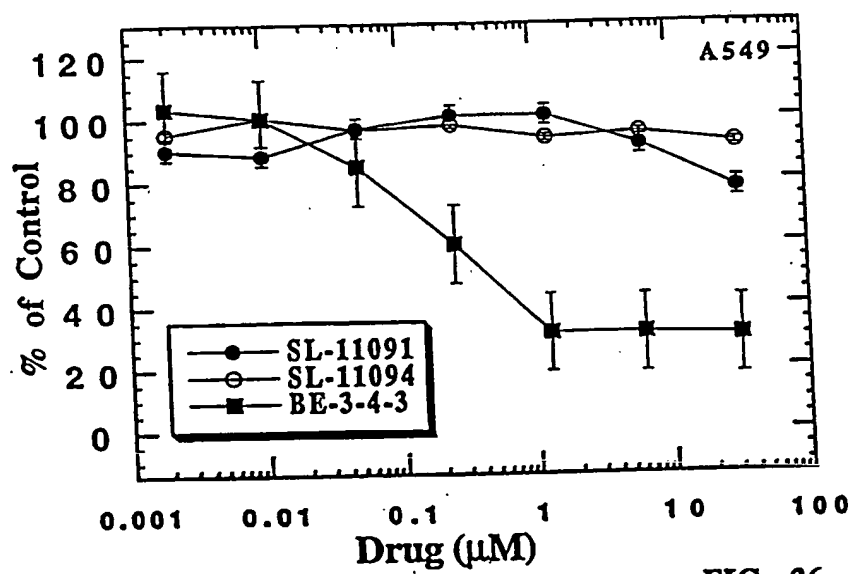


FIG. 26

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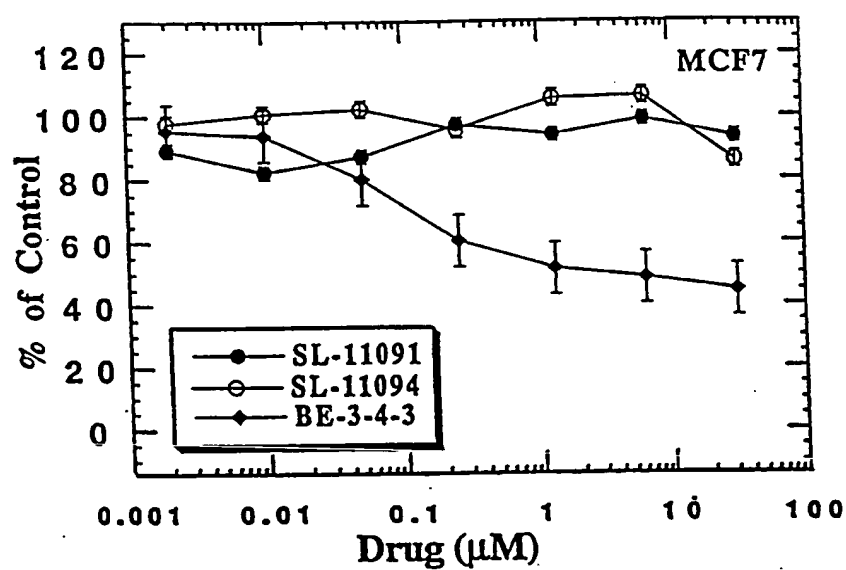


FIG. 27

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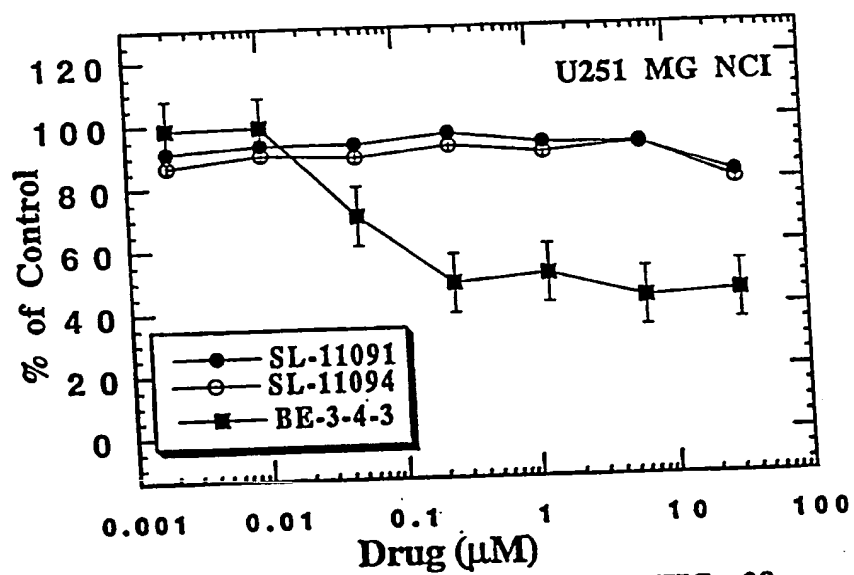
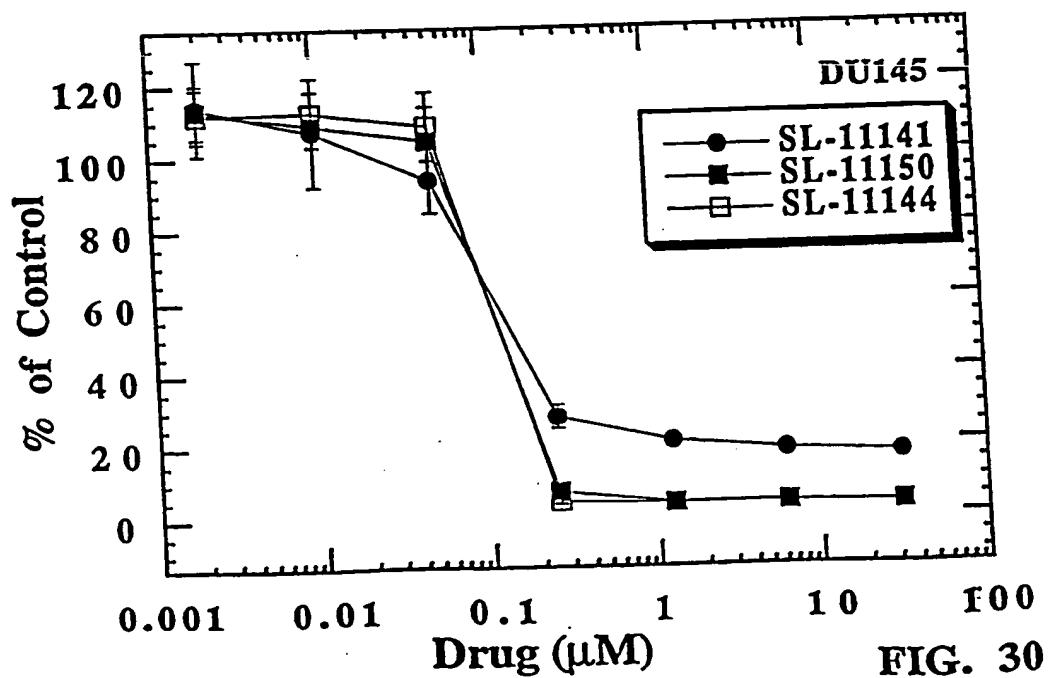
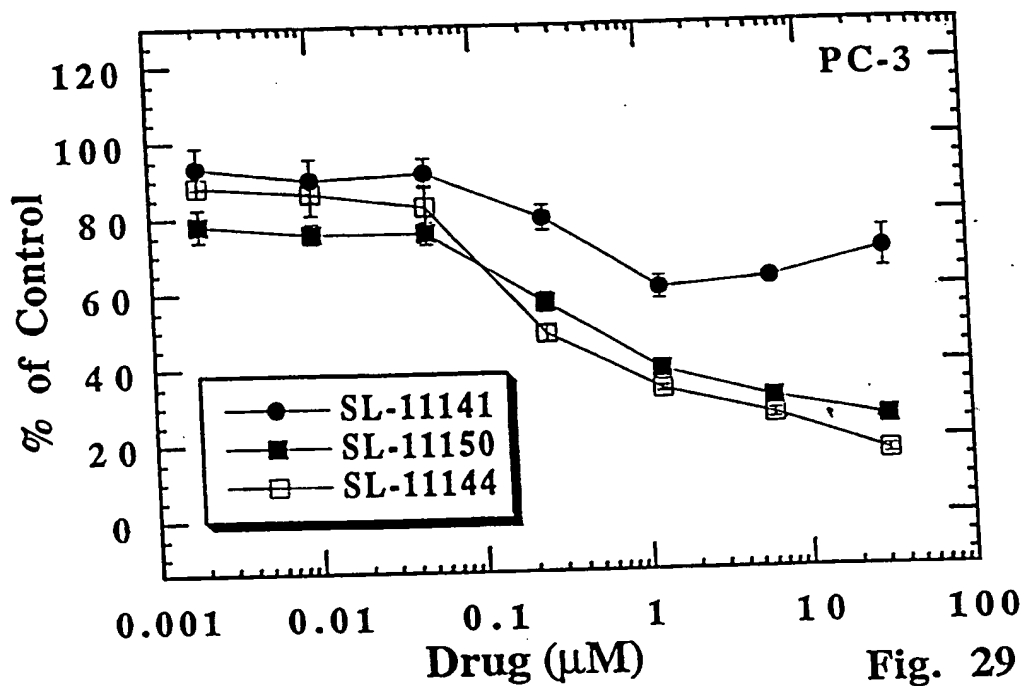
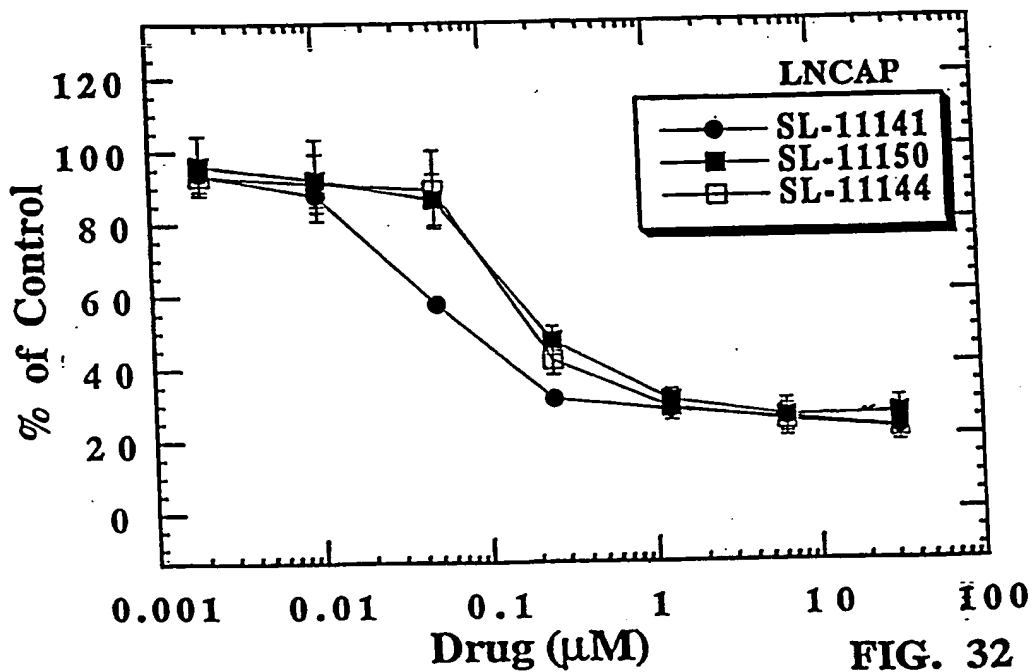
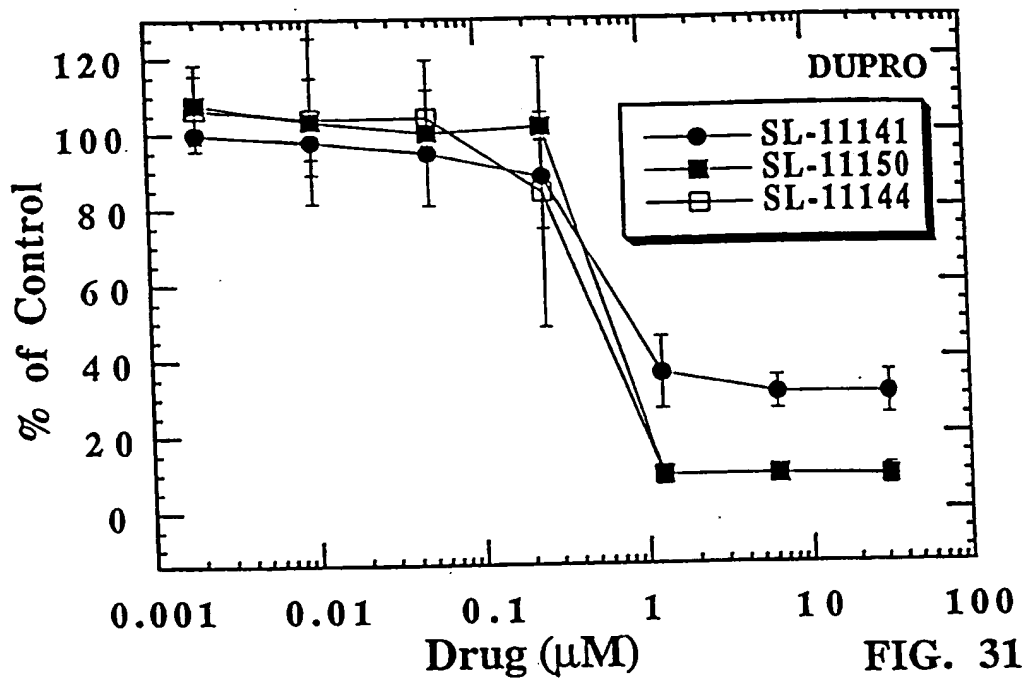


FIG. 28

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Figure 33

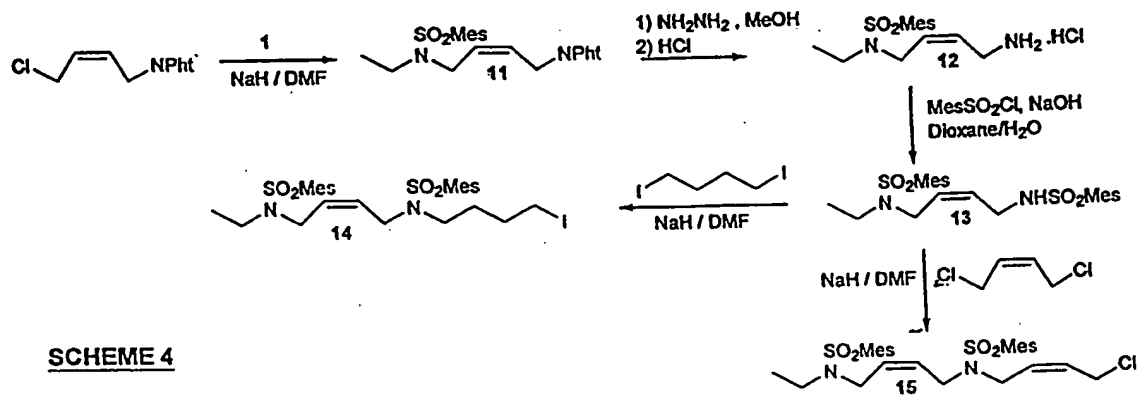
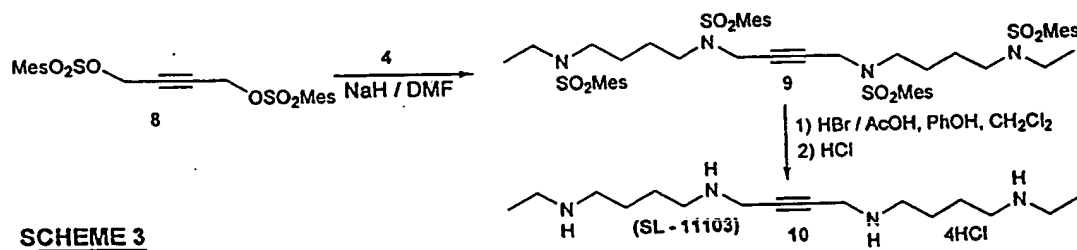
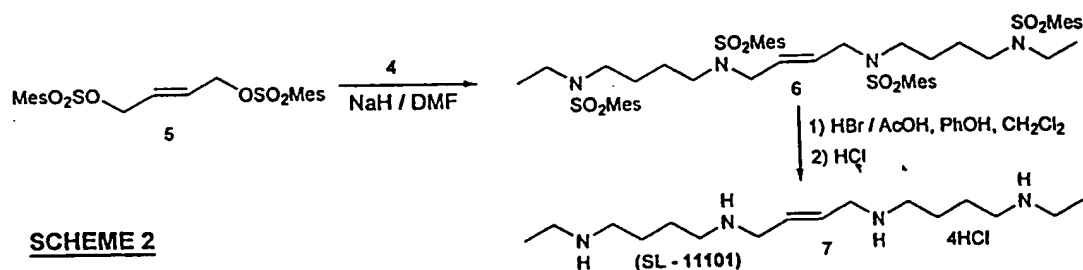
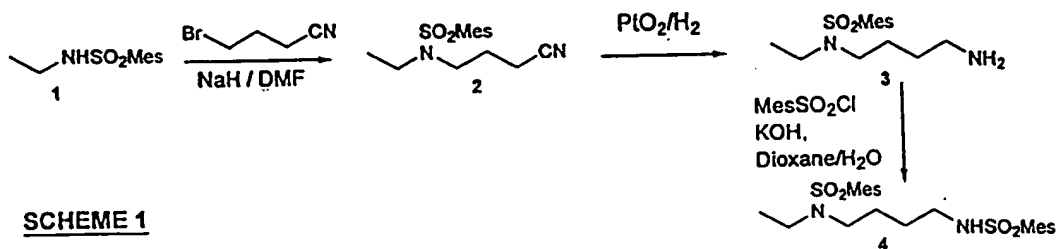
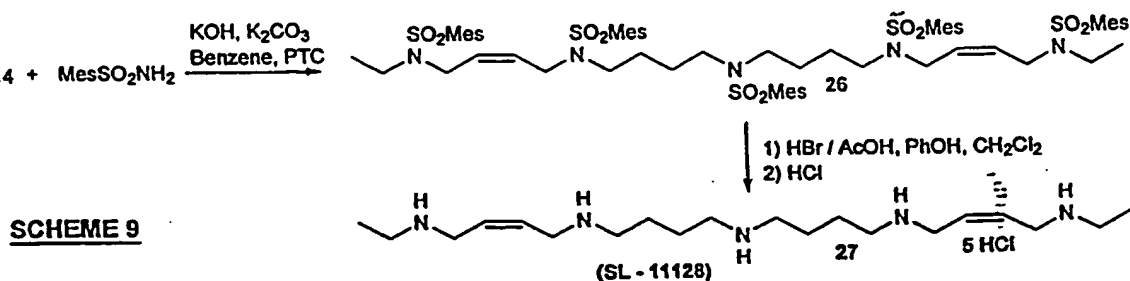
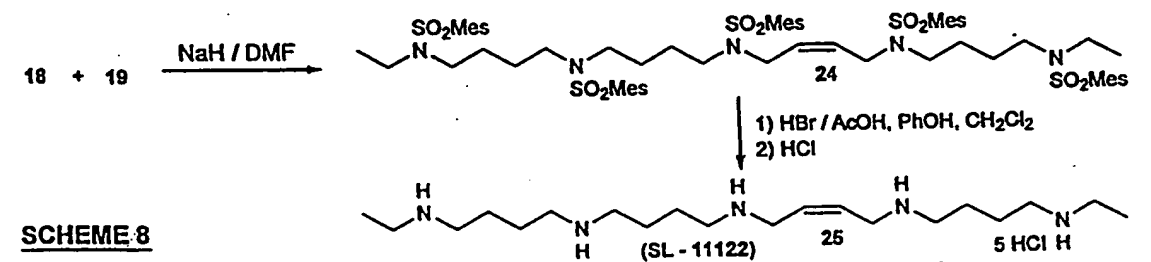
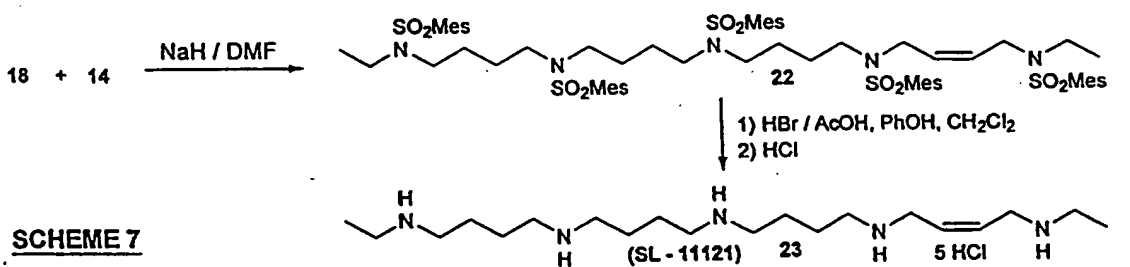
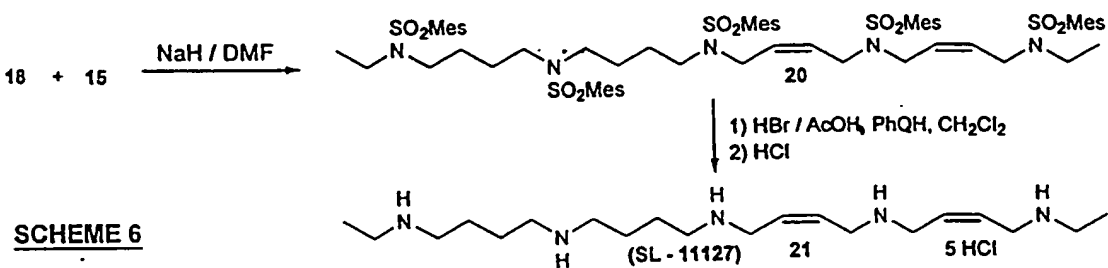
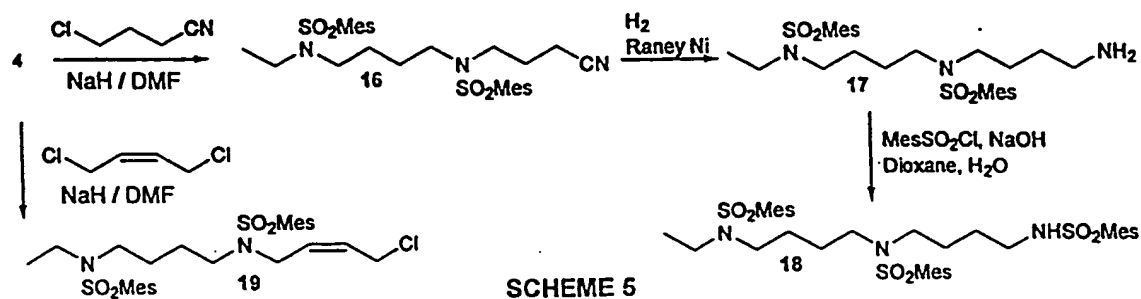


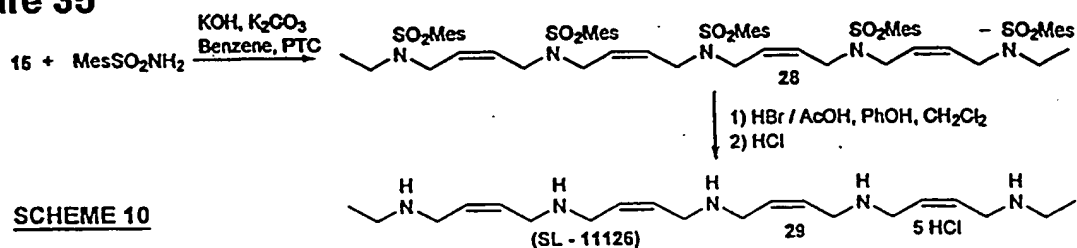
Figure 34

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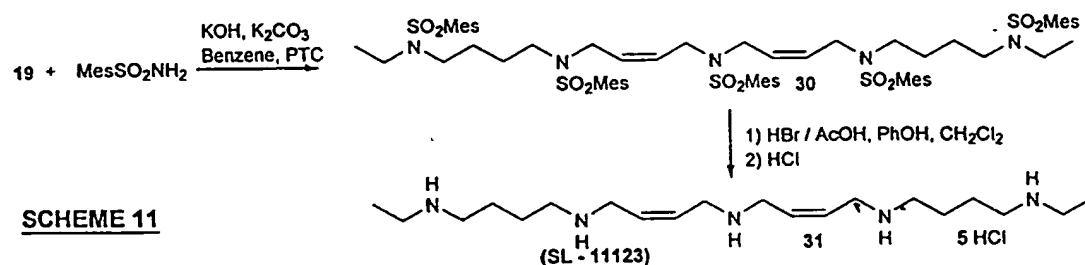


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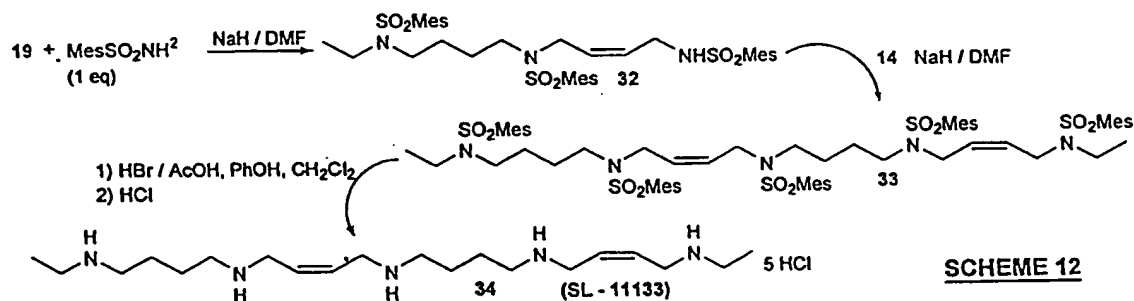
Figure 35



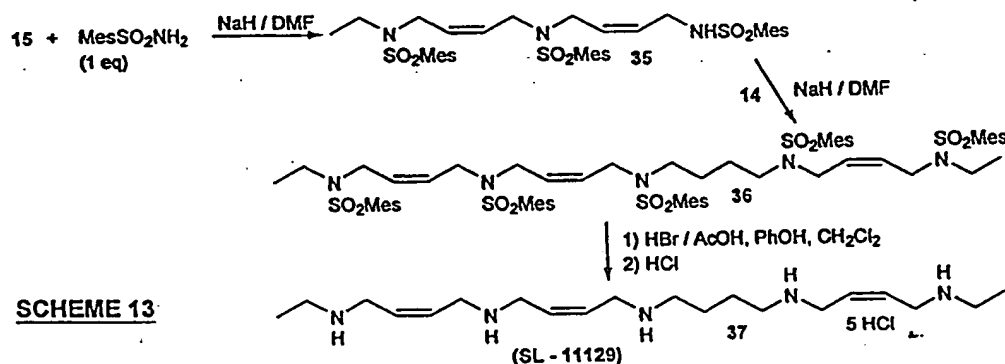
SCHEME 10



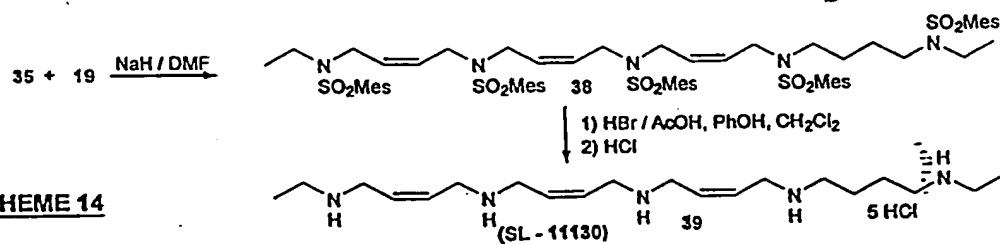
SCHEME 11



SCHEME 12



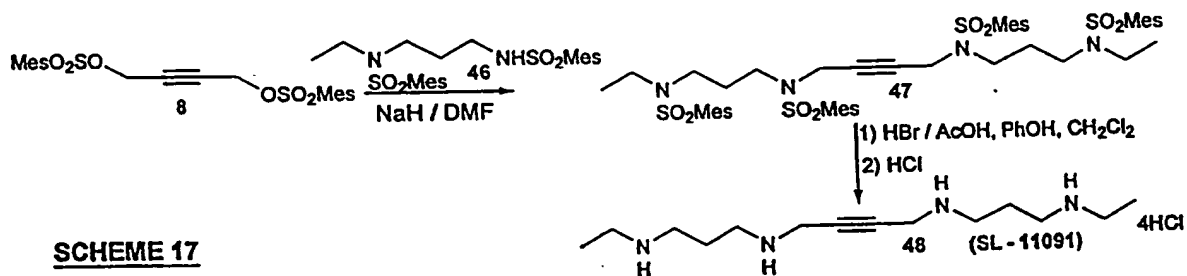
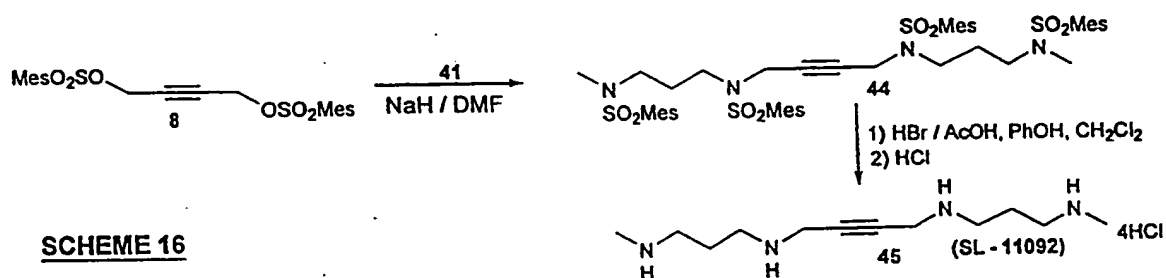
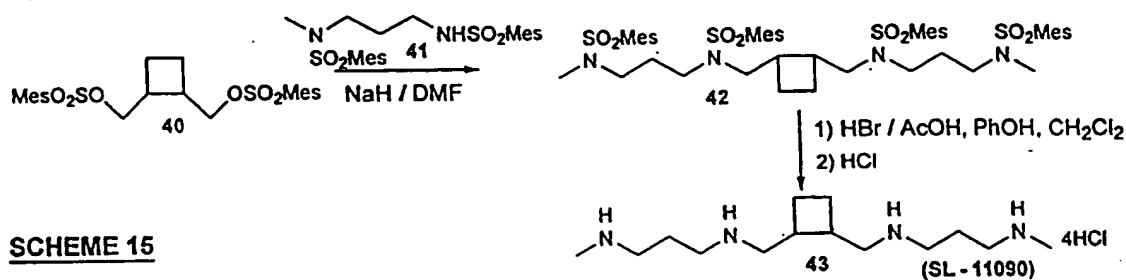
SCHEME 13



SCHEME 14

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Figure 36



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Figure 37

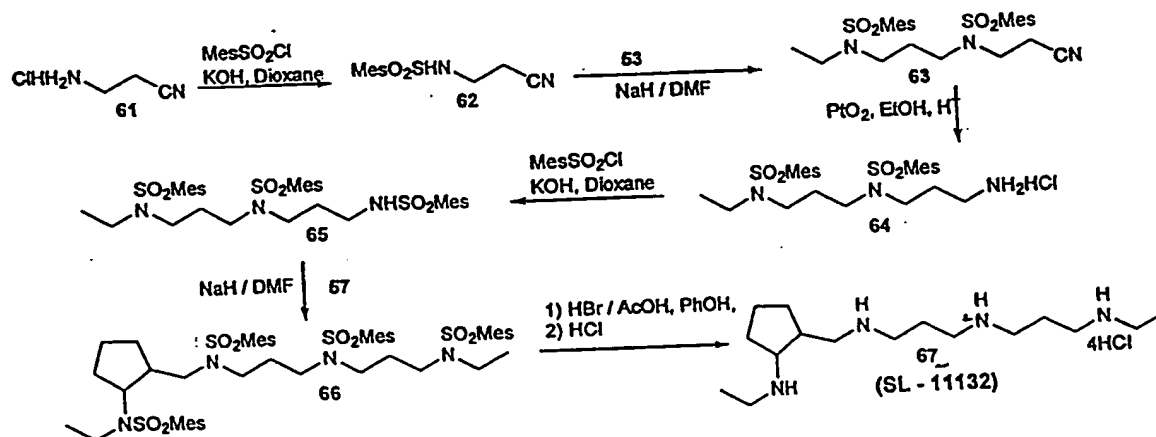
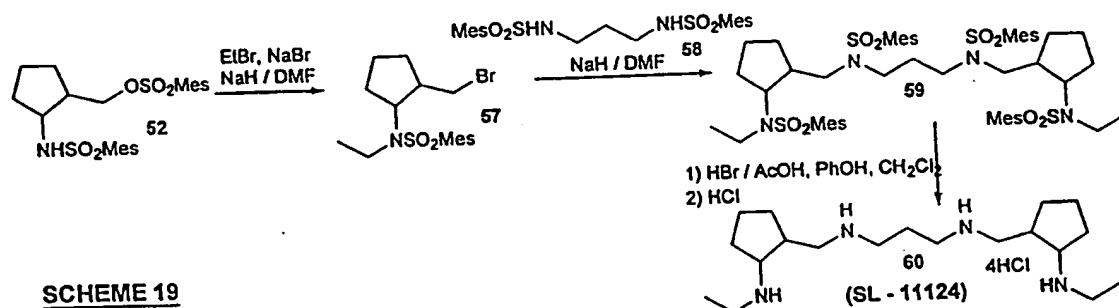
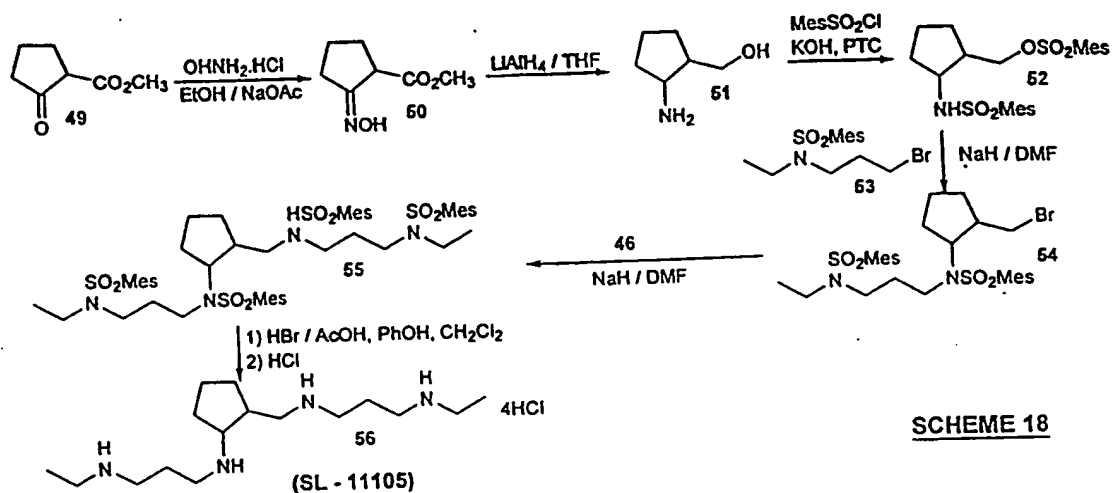
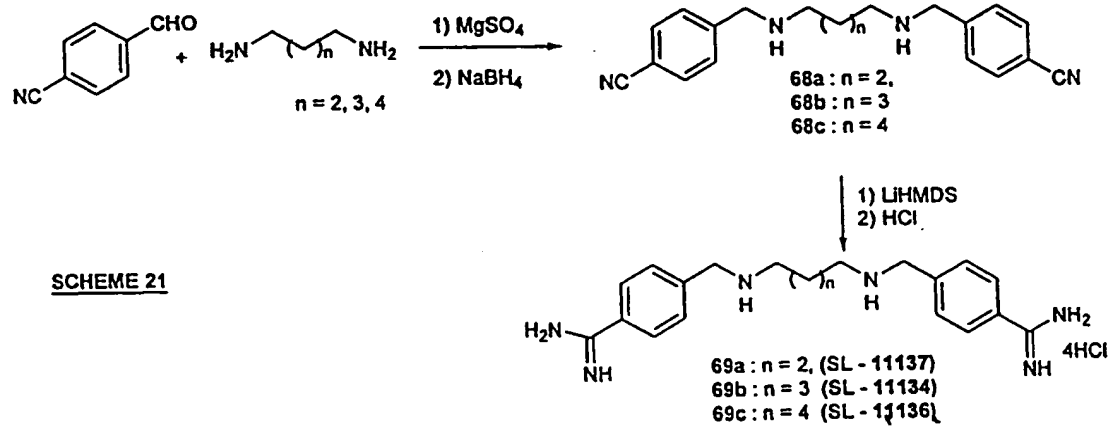


Figure 38

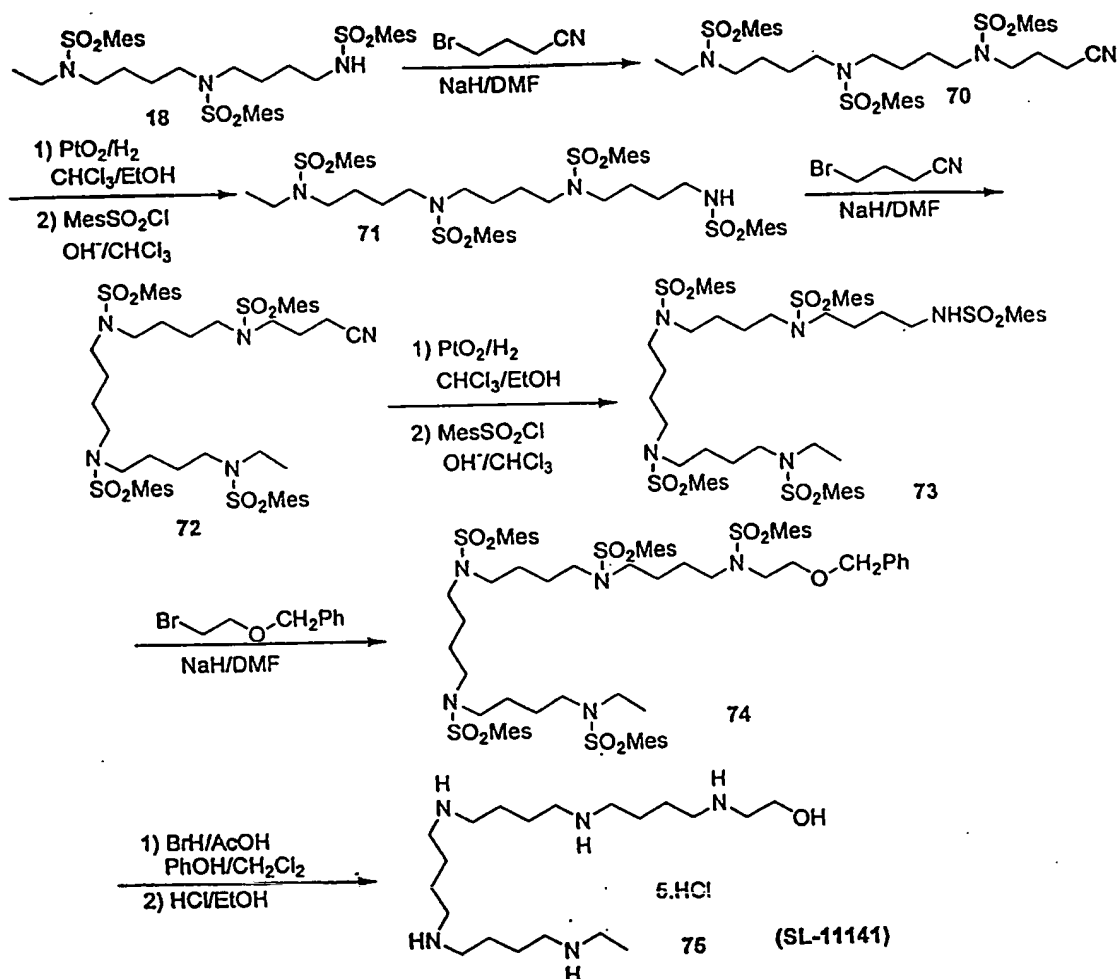
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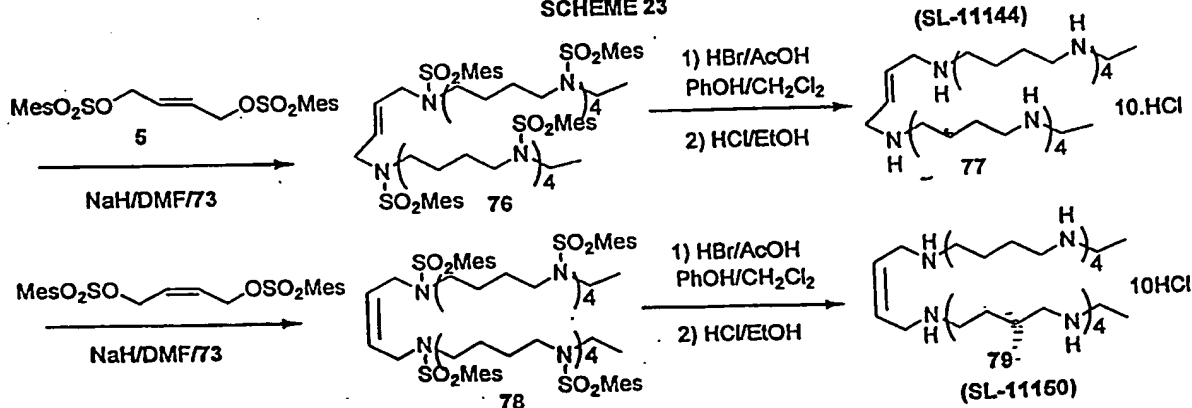
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Figure 39

SCHEME 22



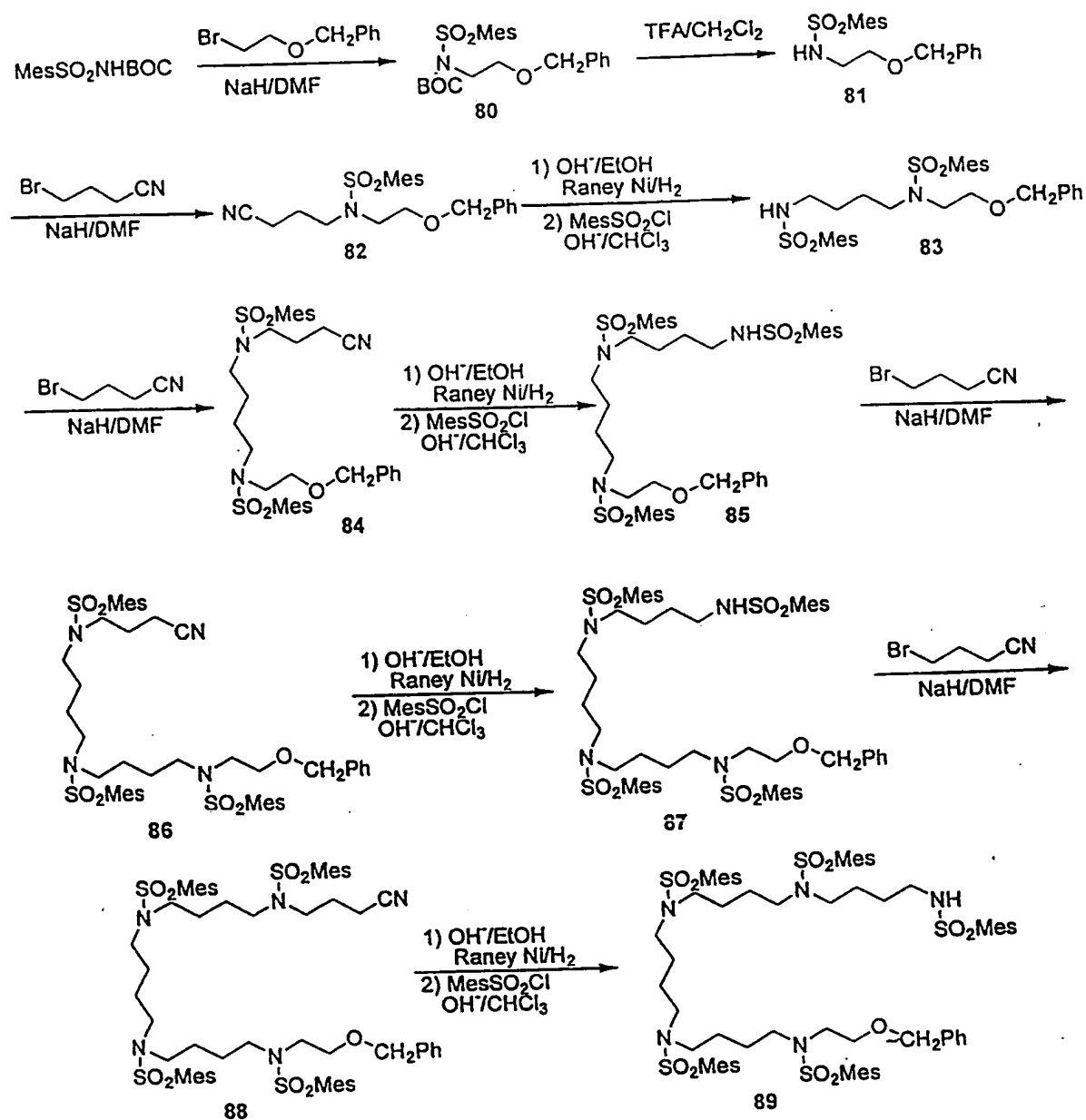
SCHEME 23



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Figure 40A

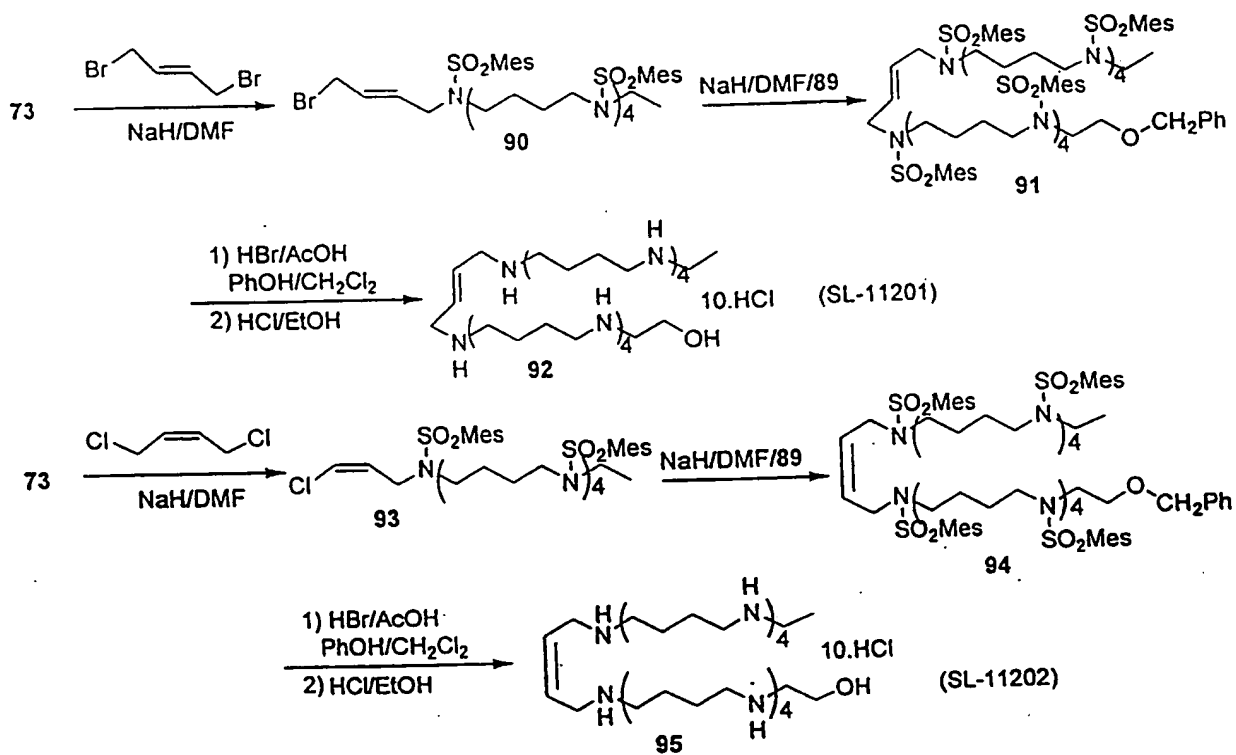
SCHEME 24



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Figure 40B

SCHEME 24 (continued)



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Scheme 501

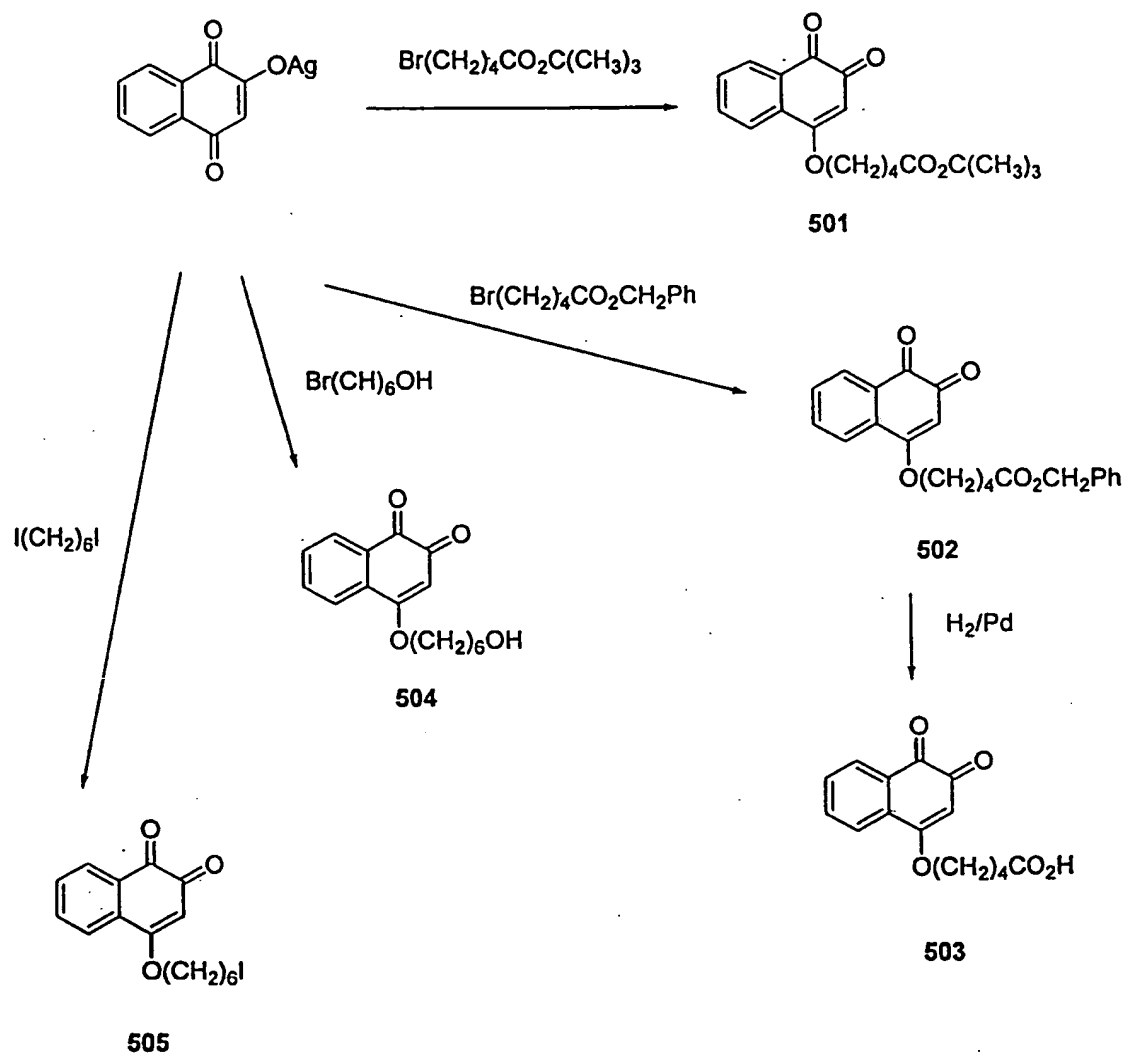


FIGURE 41

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Scheme 502

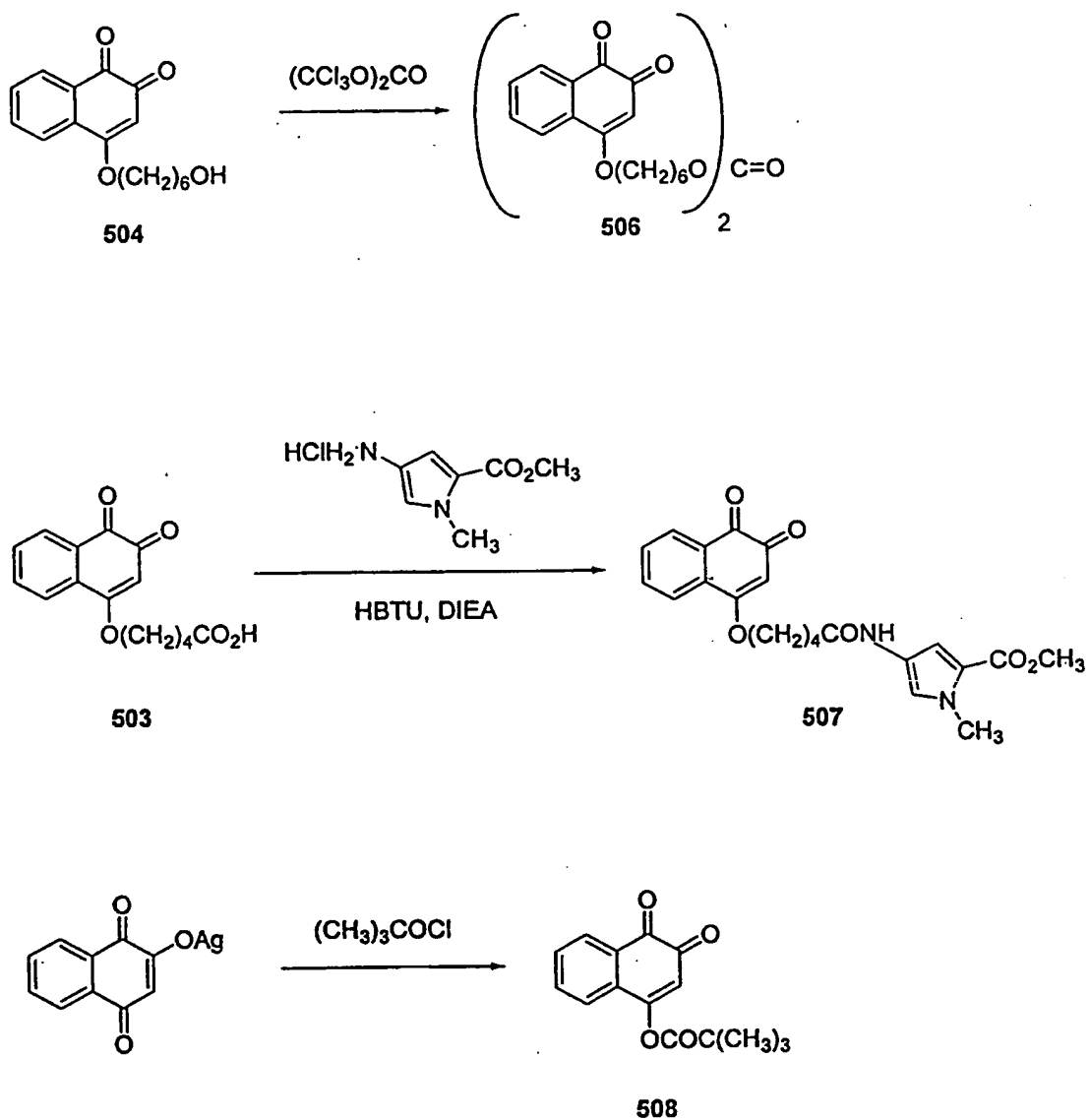


FIGURE 42

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Scheme 503

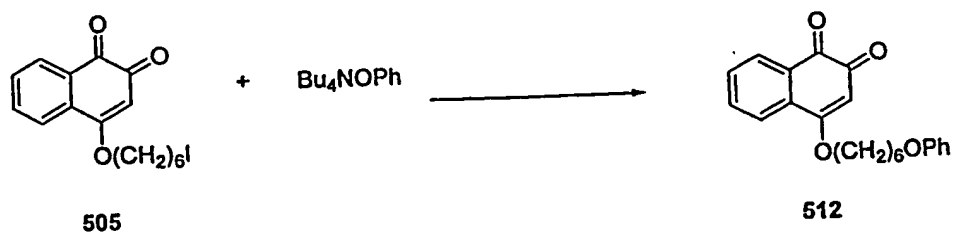
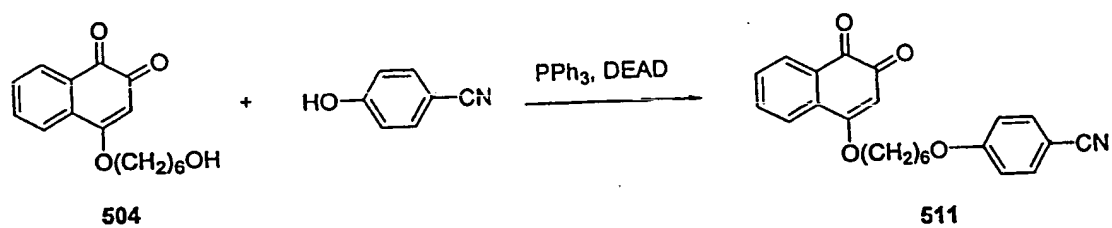
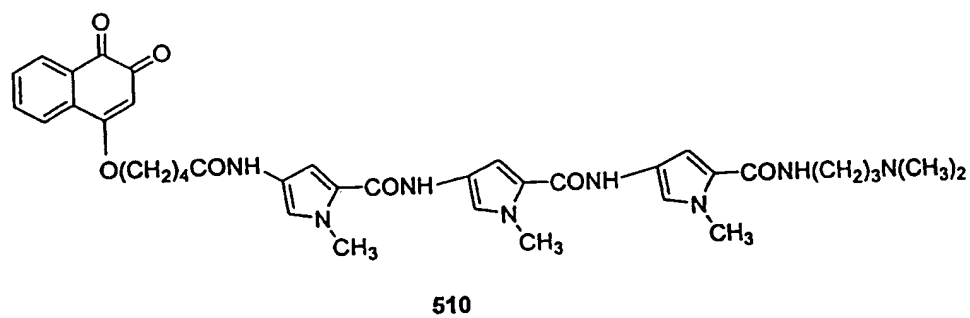
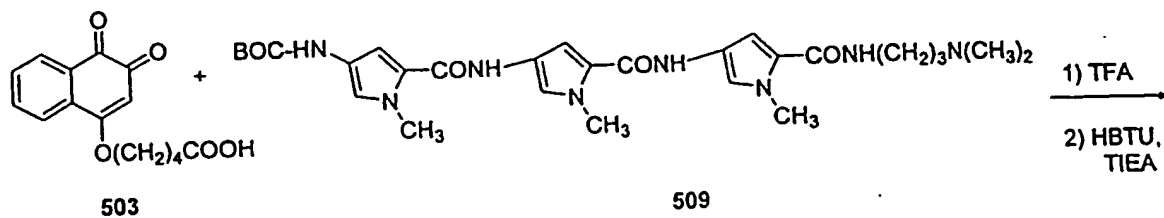


FIGURE 43

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Scheme 504

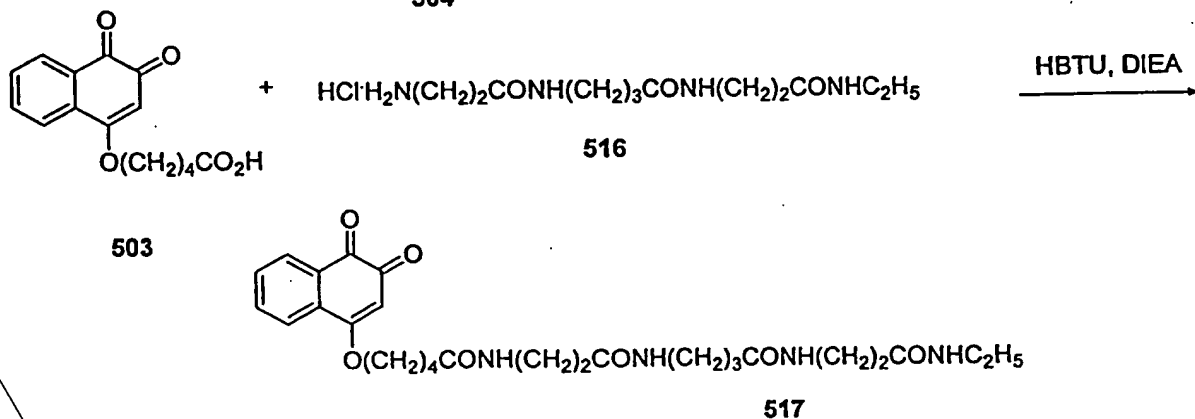
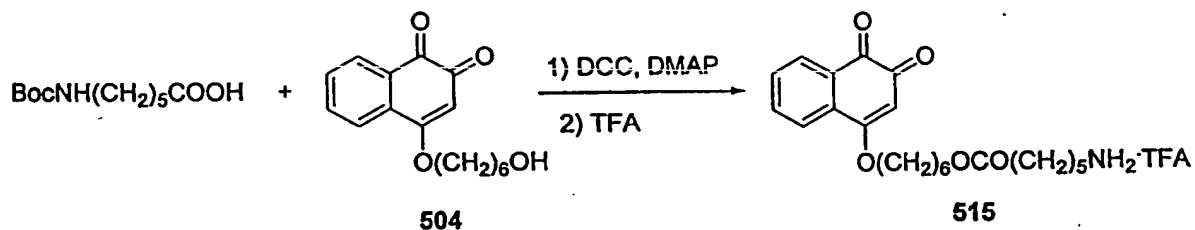
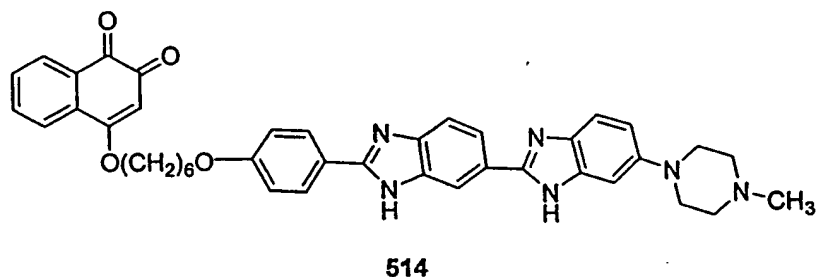
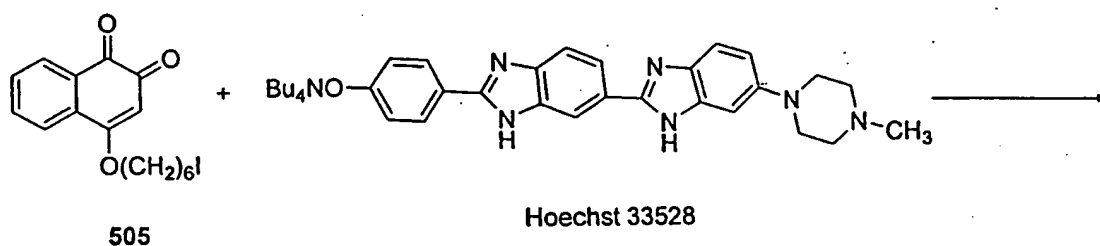
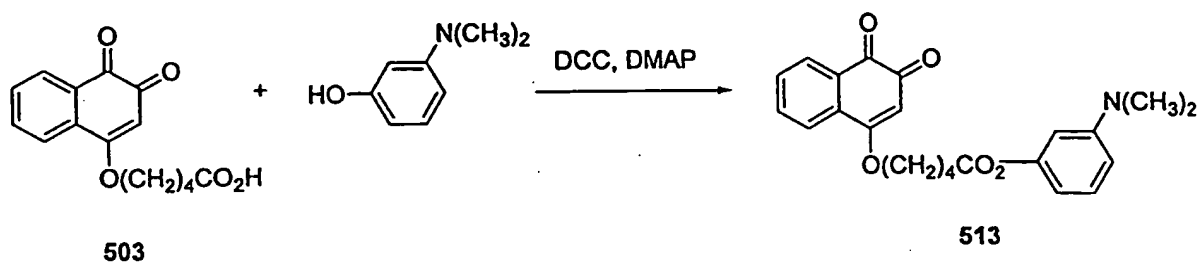


FIGURE 44

SUBSTITUTE SHEET (RULE 26)

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Scheme 505

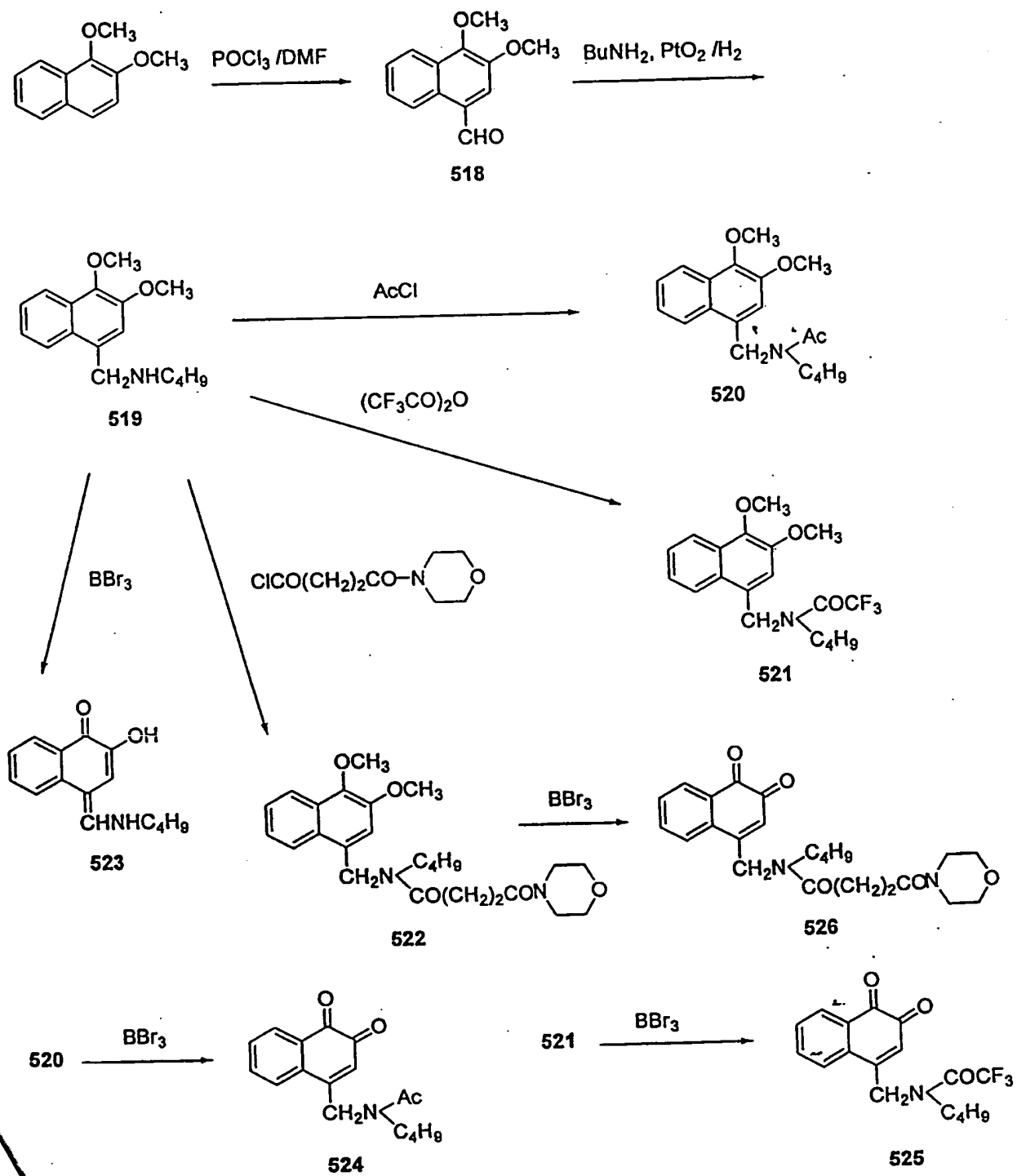


FIGURE 45

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Scheme 506

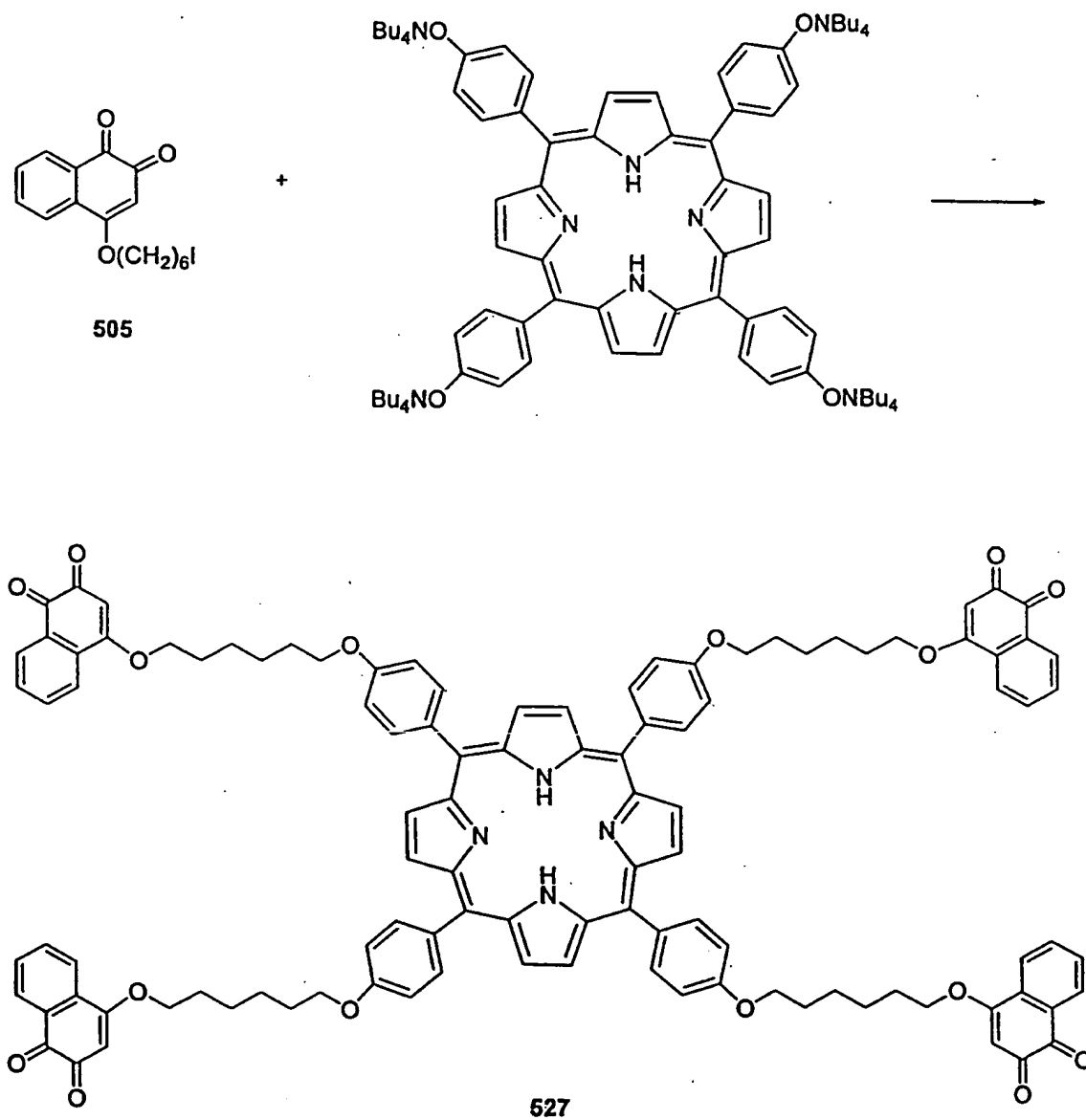


FIGURE 46

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Scheme 507

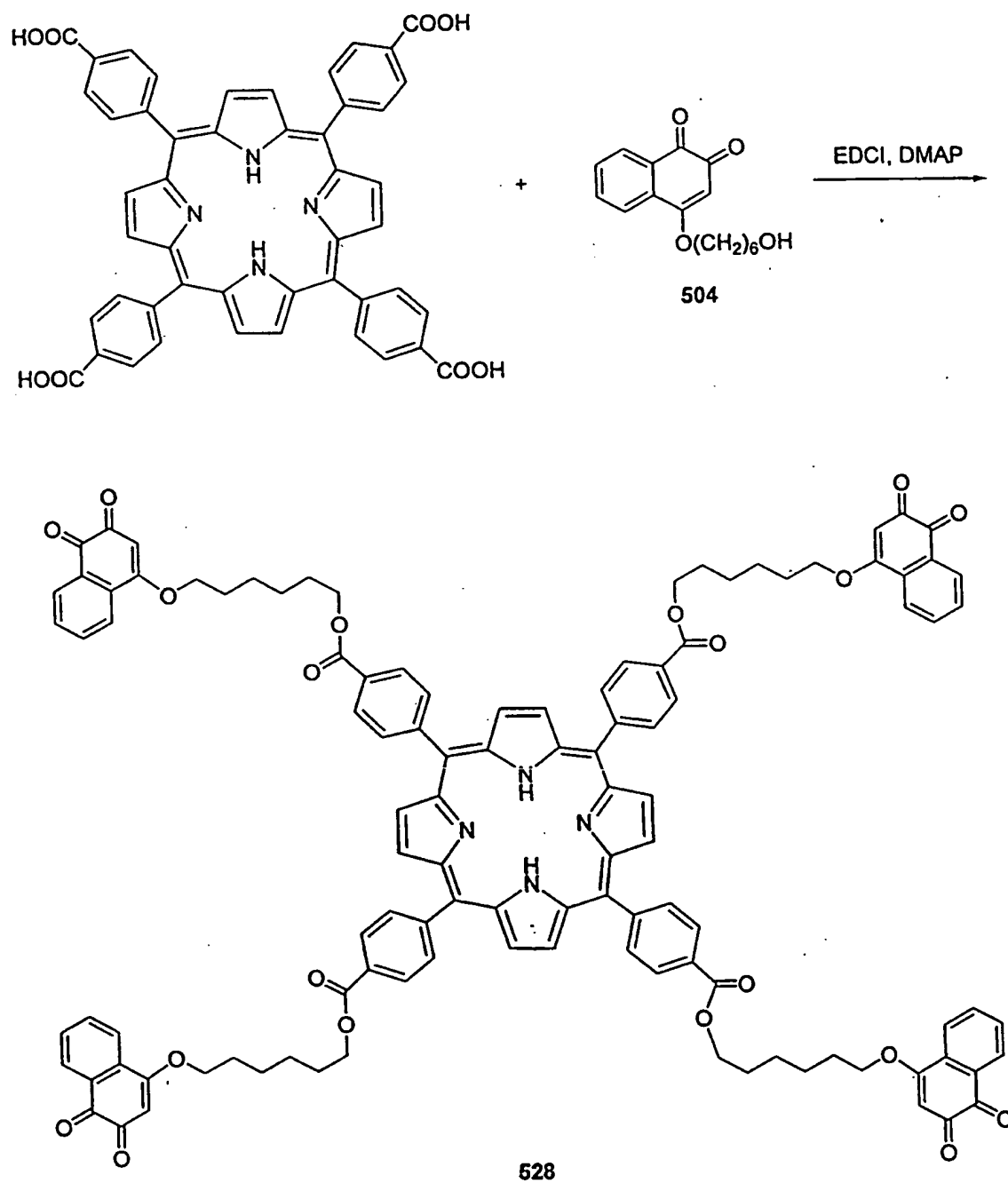


FIGURE 47

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Scheme 508

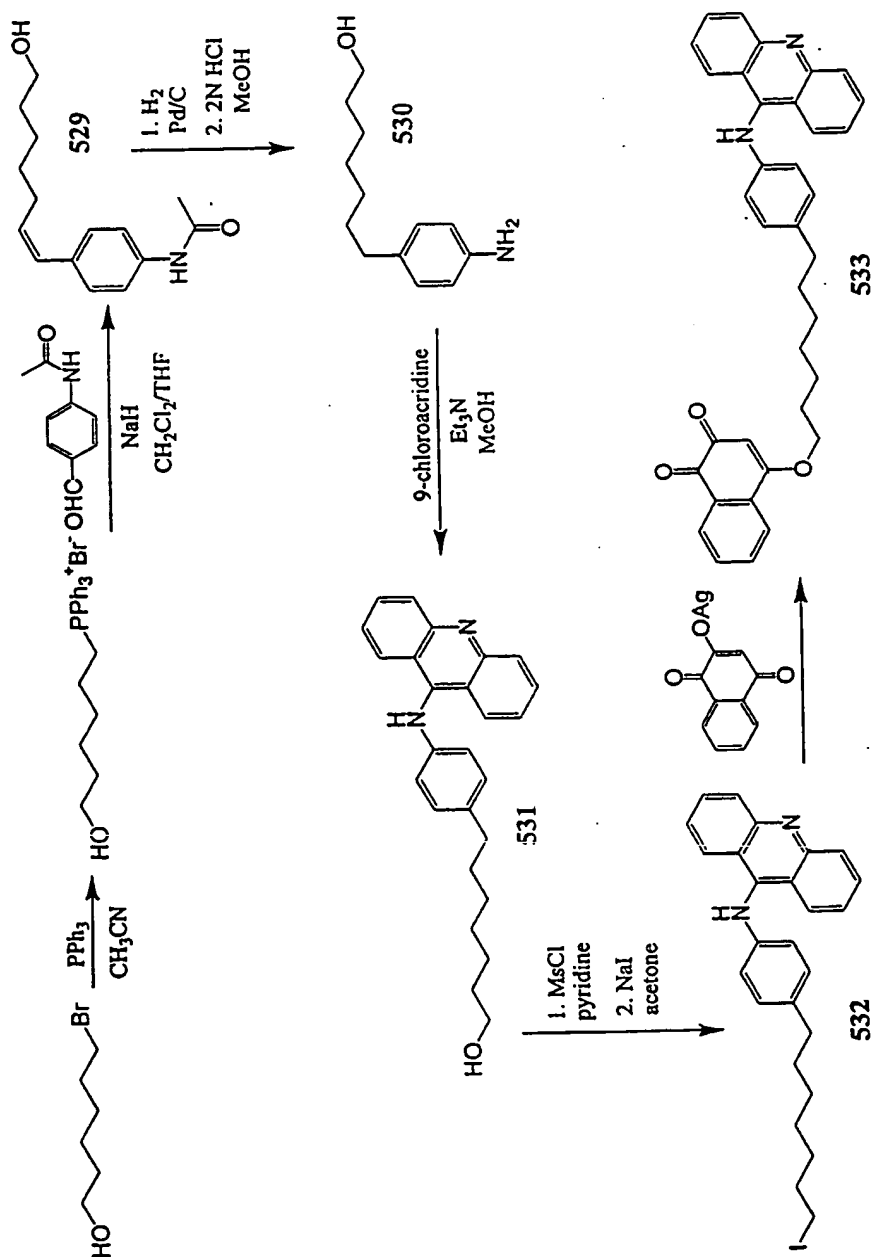


FIGURE 48

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Scheme 509

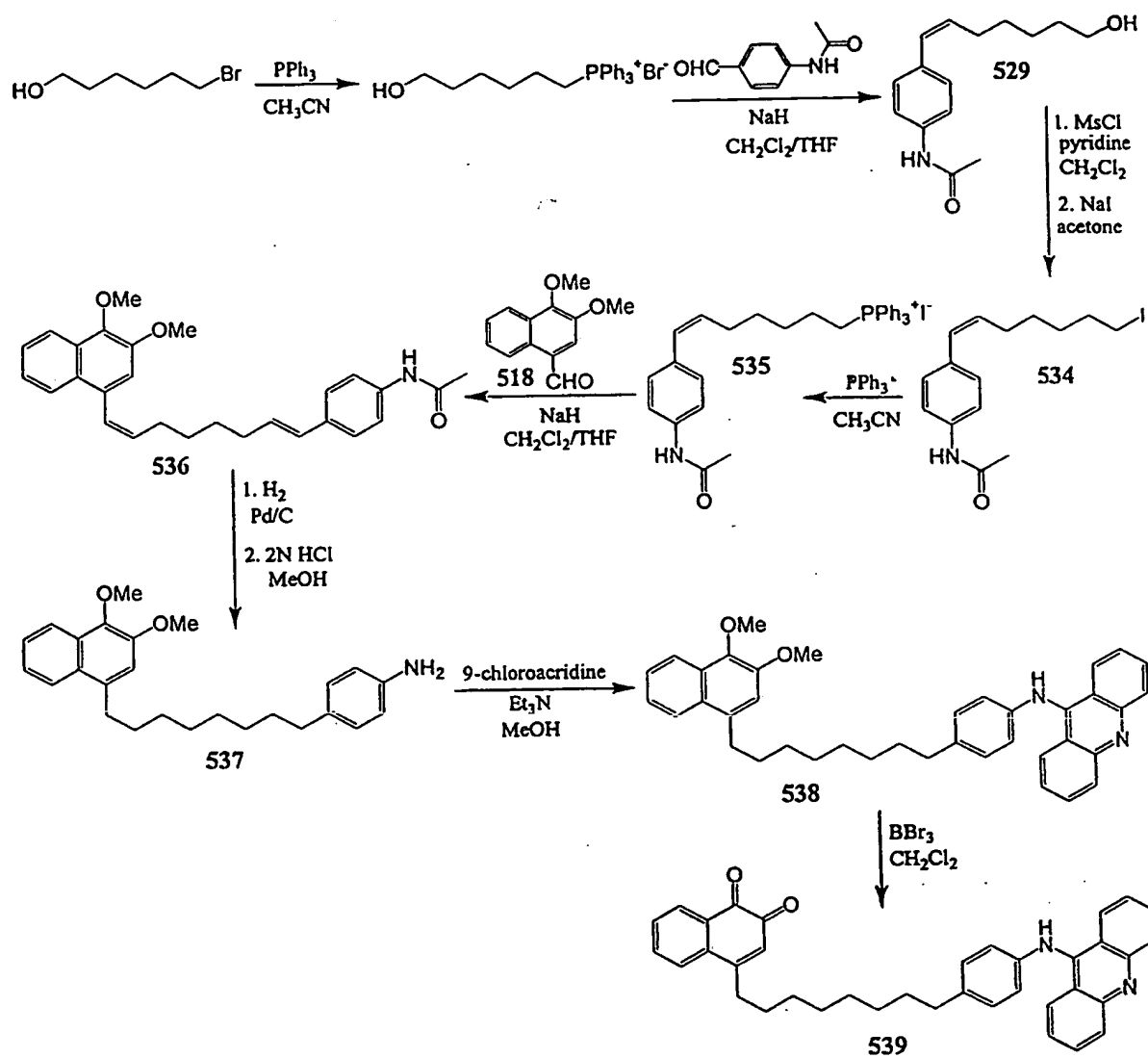


FIGURE 49

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Scheme 510

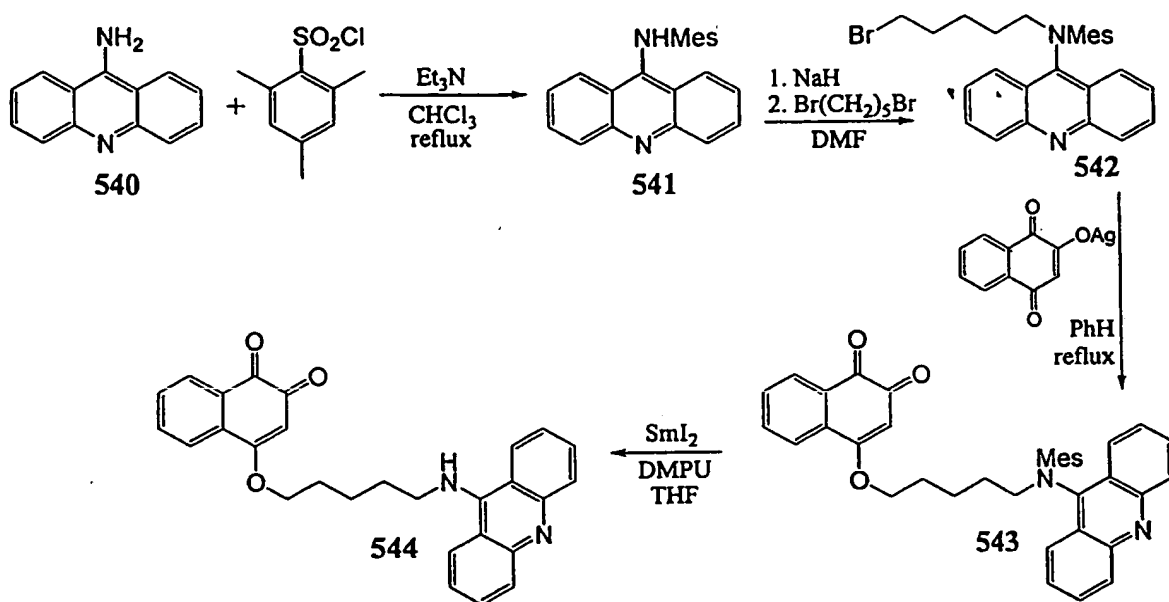


FIGURE 50

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Scheme 511

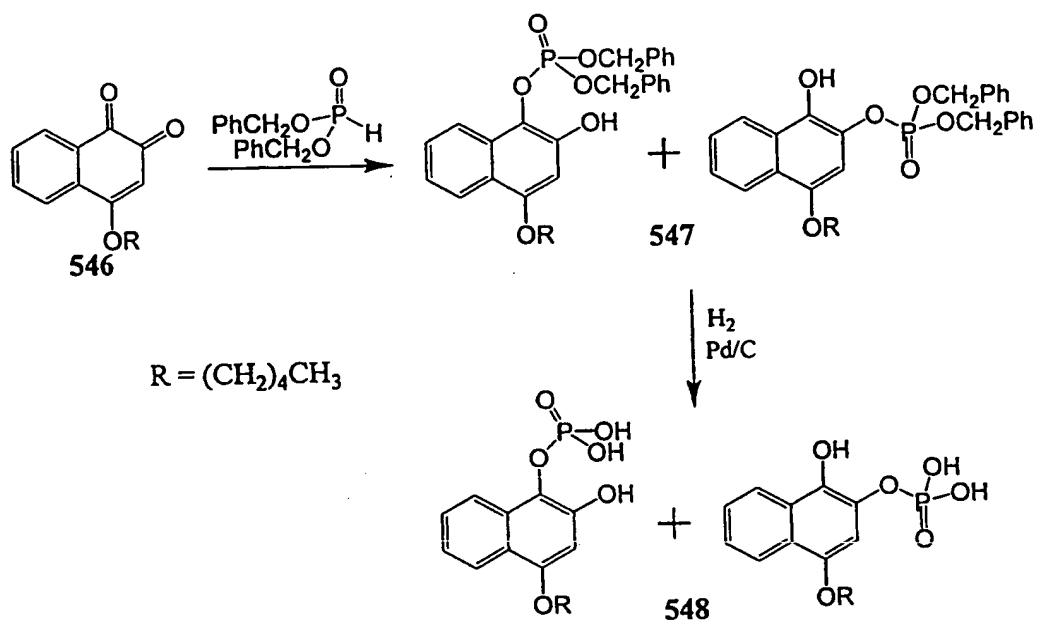


FIGURE 51

Scheme 512

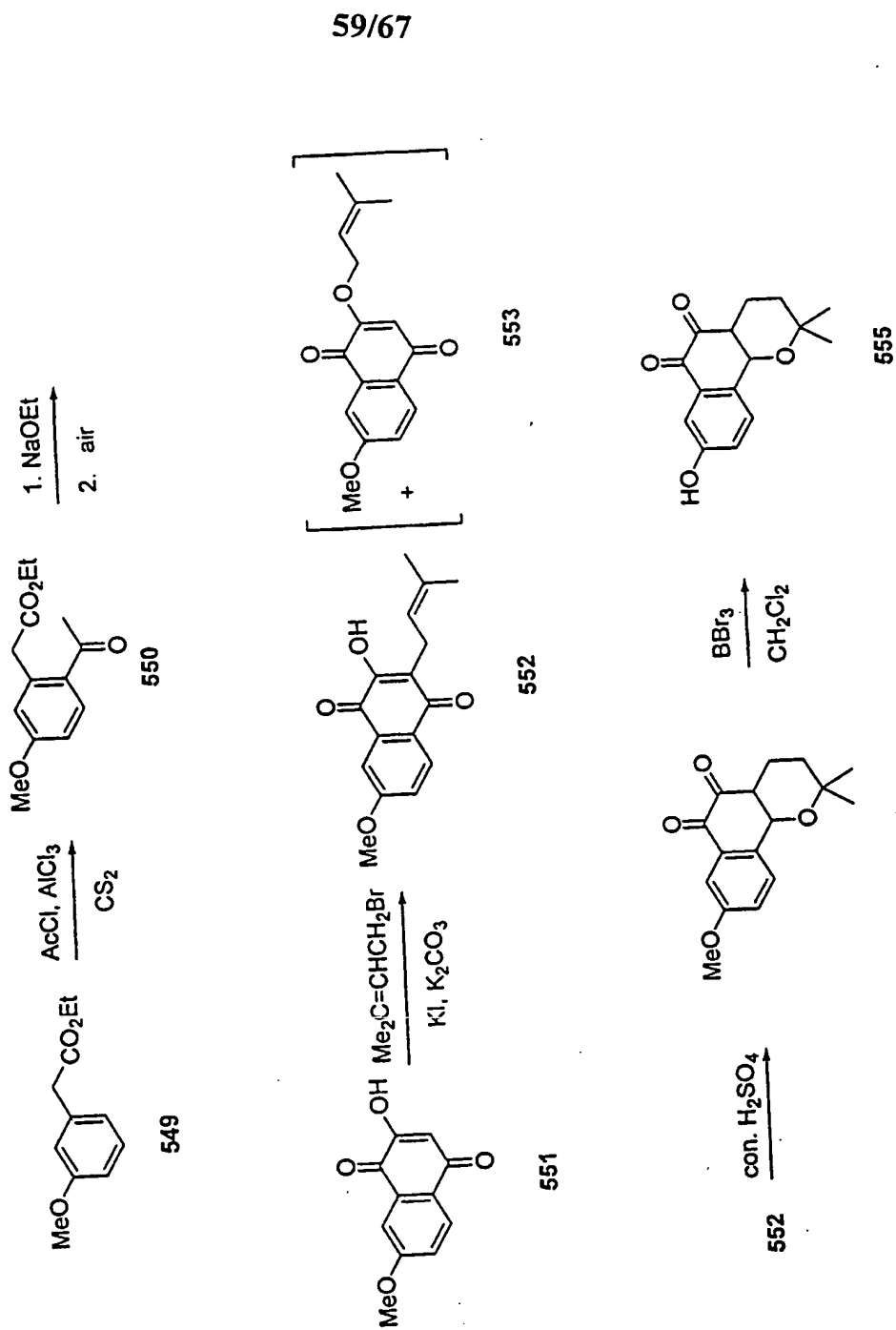


FIGURE 52

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Scheme 513

Boc-Gln + β -Ala- β -Lapachone \rightarrow Boc-Gln- β -Ala- β -Lapachone \rightarrow

Gln- β -Ala- β -Lapachone \rightarrow Boc-Leu-Gln- β -Ala- β -Lapachone \rightarrow

Leu-Gln- β -Ala- β -Lapachone \rightarrow N α -Boc-Lys(N ϵ -Cbz)-Leu-Gln- β -Ala- β -
Lapachone

\rightarrow Lys(N ϵ -Cbz)-Leu-Gln- β -Ala- β -Lapachone \rightarrow

morpholino-Ser(OBn)-Lys(N ϵ -Cbz)-Leu-Gln- β -Ala- β -Lapachone \rightarrow

morpholino-Ser-Lys-Leu-Gln- β -Ala- β -Lapachone

N-Fmoc-Ser(OBn) \rightarrow N-Fmoc-Ser(OBn)-OtBu \rightarrow Ser(OBn)-OtBu \rightarrow
morpholino-Ser(OBn)-OtBu \rightarrow morpholino-Ser(OBn)

FIG. 53

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Scheme 514

Boc-Leu + β -Lapachone \rightarrow Boc-Leu- β -Lapachone \rightarrow Leu- β -Lapachone \rightarrow

Boc-Gln-Leu- β -Lapachone \rightarrow Gln-Leu- β -Lapachone \rightarrow

Boc-Leu-Gln-Leu- β -Lapachone \rightarrow Leu-Gln-Leu- β -Lapachone \rightarrow

Boc-Lys(N ϵ -Cbz)-Leu-Gln-Leu- β -Lapachone \rightarrow

Lys(N ϵ -Cbz)-Leu-Gln-Leu- β -Lapachone \rightarrow

morpholino-Ser(OBn)-Lys(N ϵ -Cbz)-Leu-Gln-Leu- β -Lapachone \rightarrow

morpholino-Ser-Lys-Leu-Gln-Leu- β -Lapachone

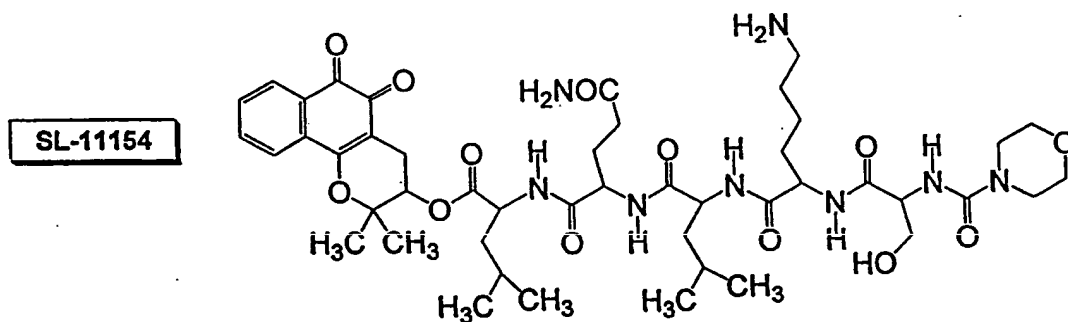


FIG. 54

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Scheme 25

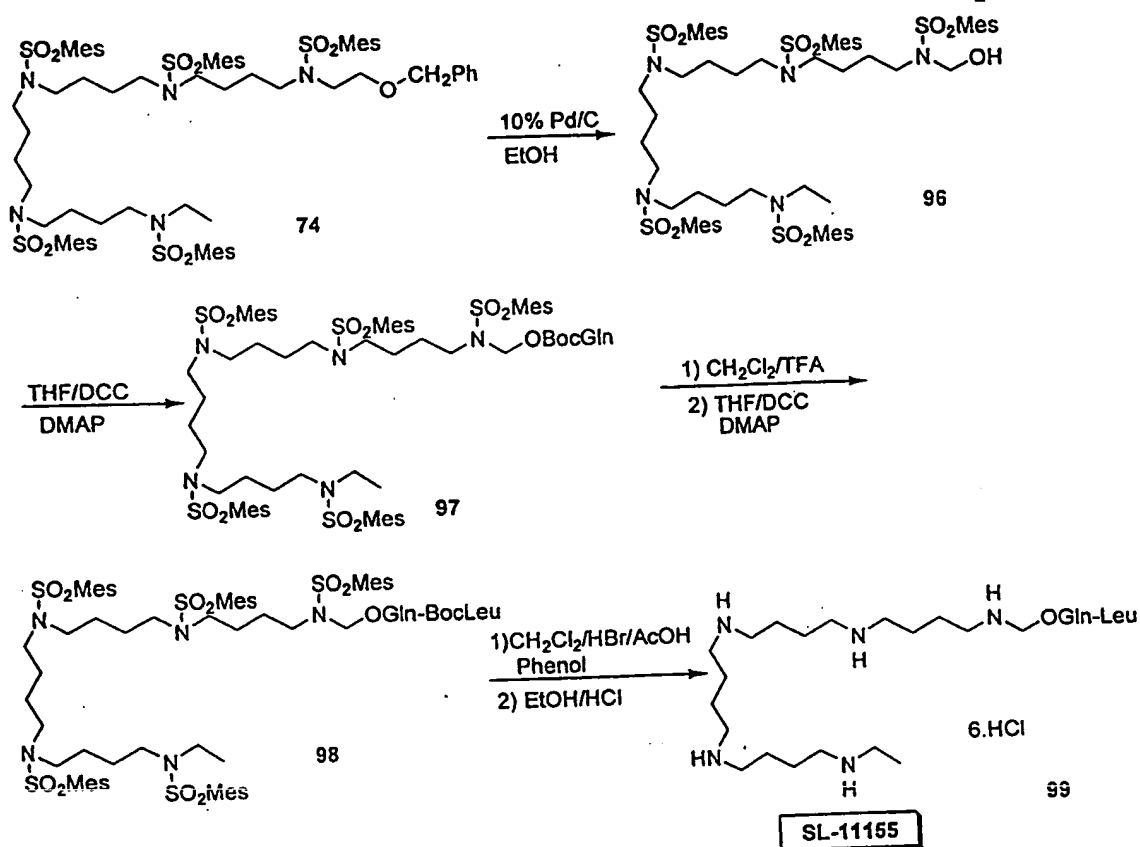
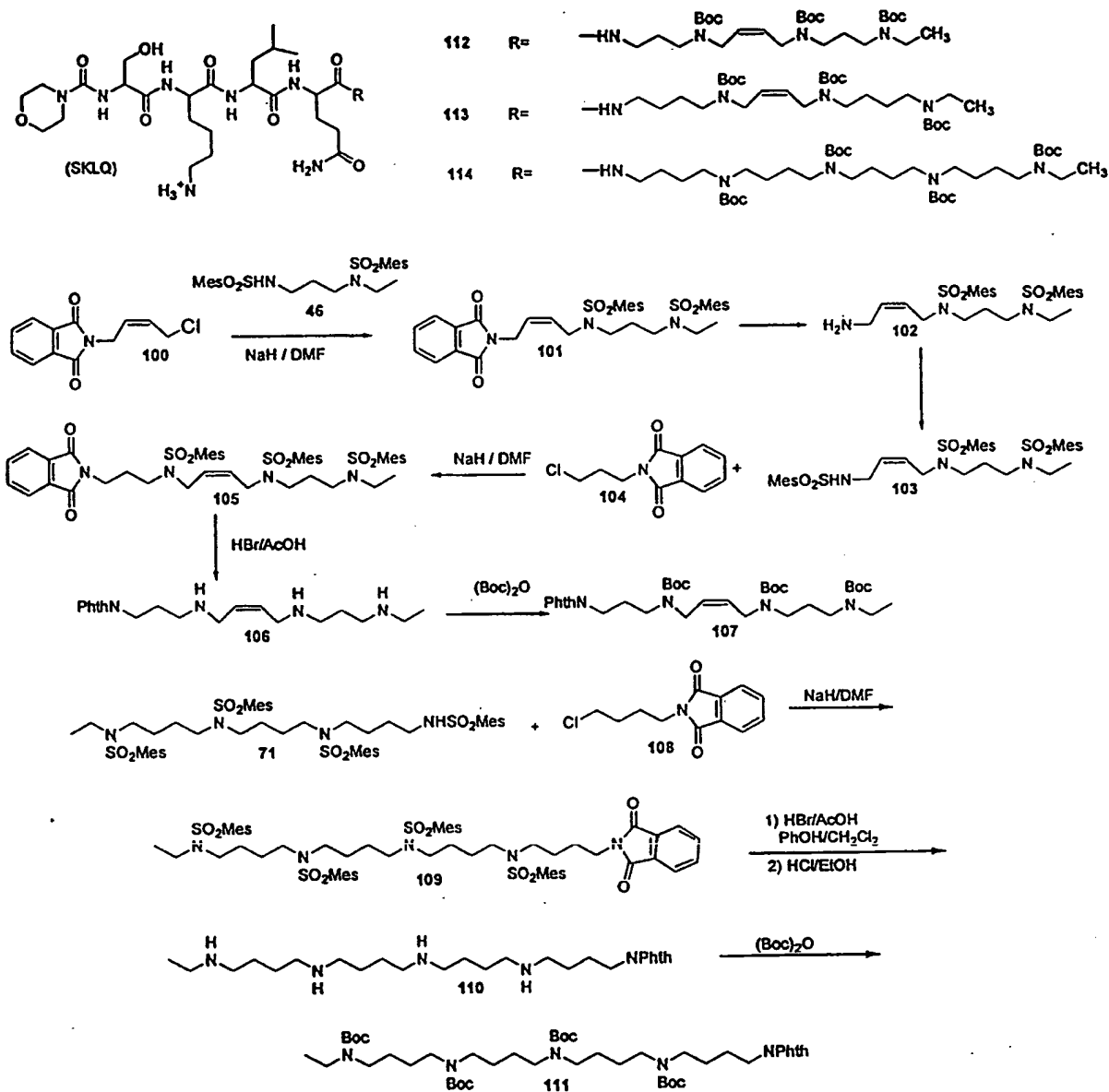


Figure 55

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SCHEME 26

Figure 56

Figure 57

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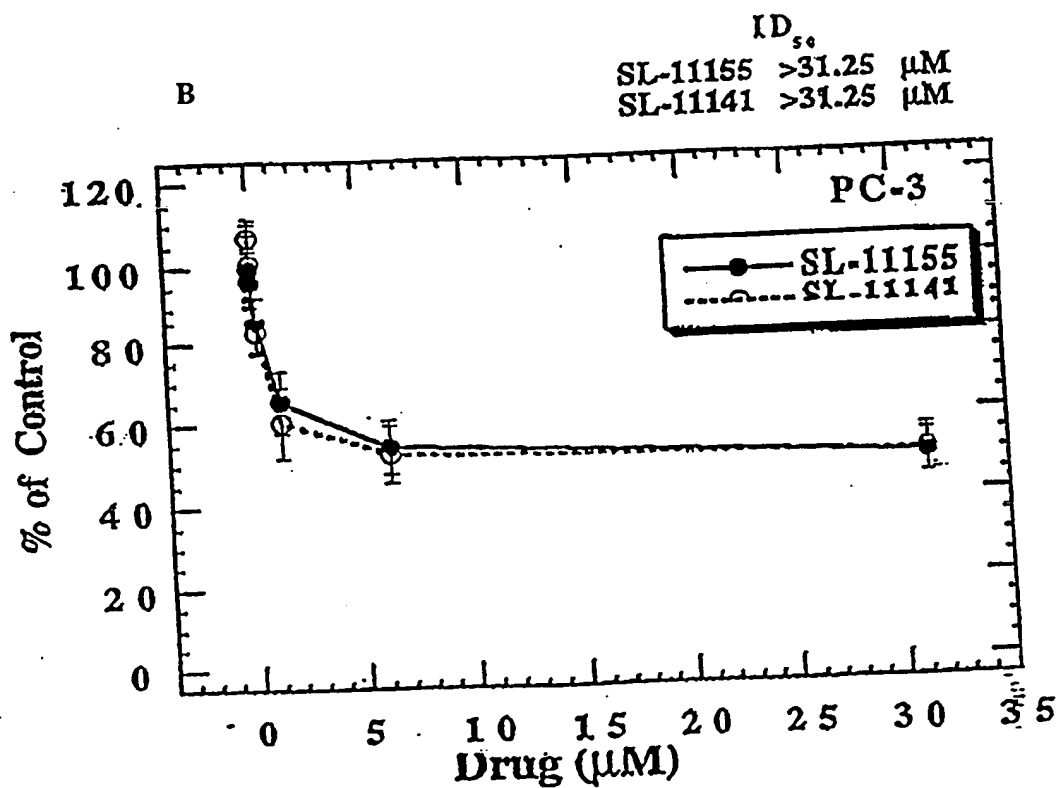
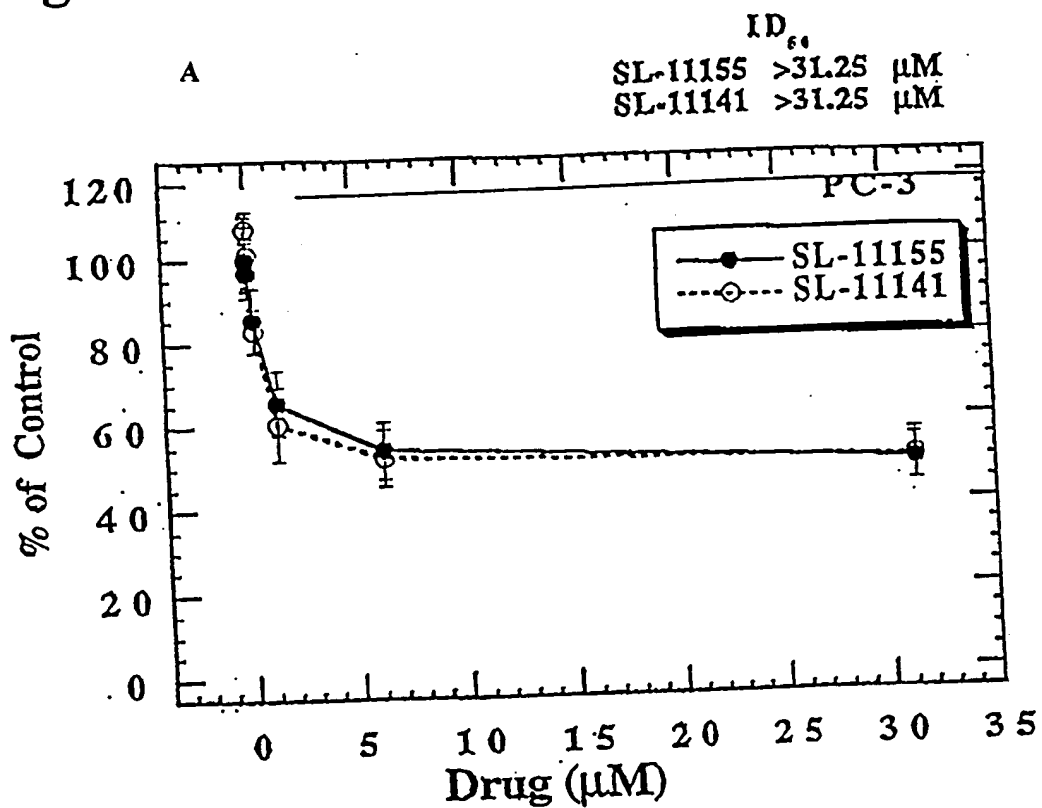


Figure 58

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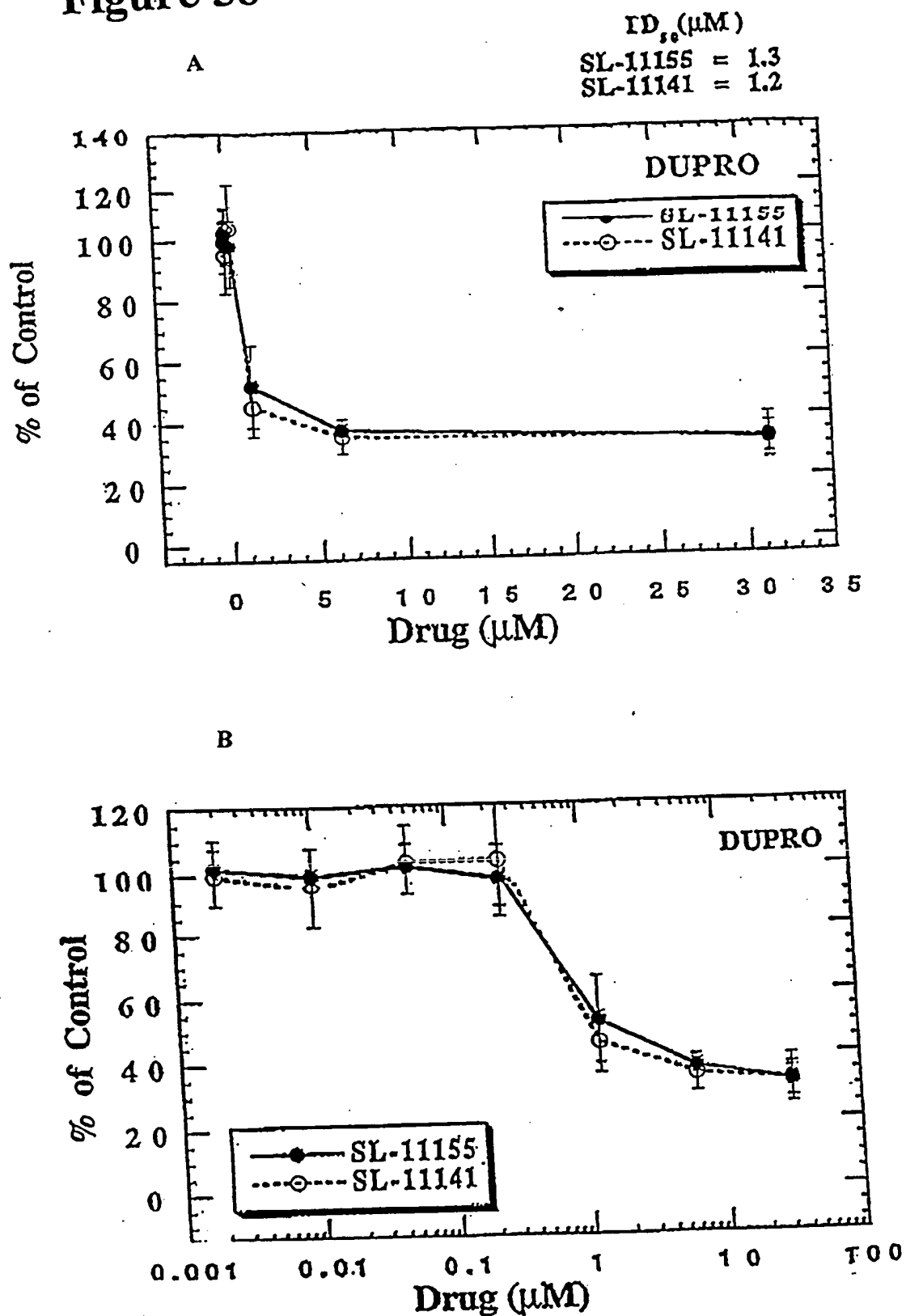


Figure 59

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ID₅₀ (μM)

SL-11155 = 0.8

SL-11141 = 0.7

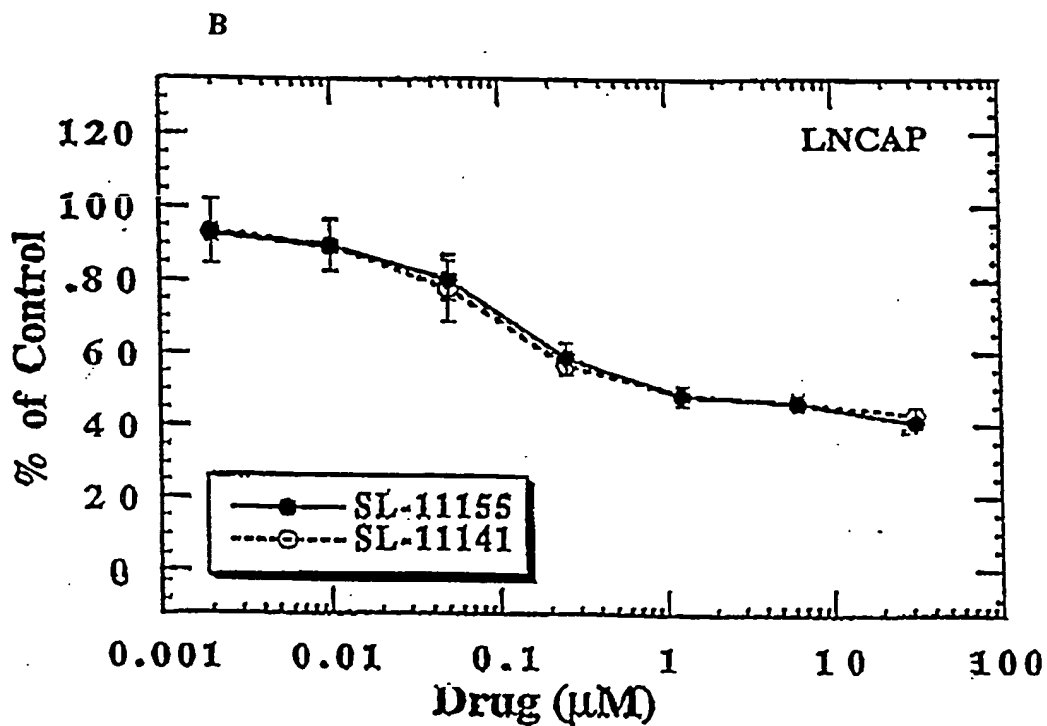
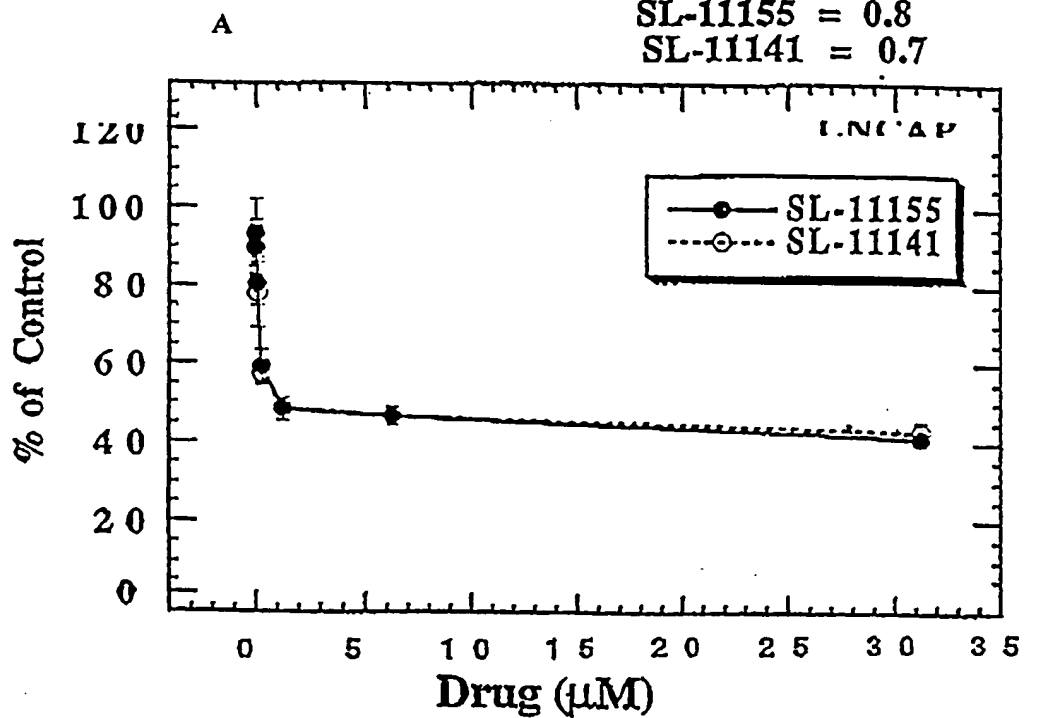


Figure 60

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